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13. ABSTRACT *(Maximum 200 Words)*

The Center for Integration of Medicine and Innovative Technology (CIMIT), is a consortium of nonprofit Massachusetts-based institutions led by Massachusetts General Hospital and includes Brigham and Women's Hospital, Massachusetts Institute of Technology and Draper Laboratory. The primary aim of the Center is to develop technologies that will advance the capability of modern medicine to diagnose and treat patients using minimally invasive and less costly approaches. CIMIT will coordinate and implement research programs in cardiovascular disease, cancer, stroke, trauma and critical care, that are supported by basic science and engineering development in biomaterials, endoscopic tools, energy delivery, intelligent decision systems, medical imaging, micro-sensors, outcomes, robotics and simulation. A unique military/civilian partnership fostered by CIMIT will allow DOD technologies to be evaluated by CIMIT investigators and facilitate the transfer to the military of successful minimally invasive approaches developed at CIMIT. An educational program, which includes coursework, seminars, and on-site training opportunities, will serve the shared needs of academic and military physicians and scientists. The overall goal of CIMIT is to create a national program that combines clinical and technological excellence in order to generate, develop, and reduce-to-practice innovative and high-impact concepts in minimally invasive therapy.

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CIMIT

Innovative Technology for Medicine

MG John S. Parker (left), Commanding General, USAMRMC, after successfully placing a chest tube into the VIRGIL simulator. Dr. Steve Dawson (right), team leader of the CIMIT Simulation Group, congratulates General Parker.

Annual Progress Report
October 1, 2000 – September 30, 2001

Attachment 2

FOREWORD

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CENTER FOR INTEGRATION OF MEDICINE AND INNOVATIVE TECHNOLOGY
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CENTER FOR INTEGRATION OF MEDICINE AND INNOVATIVE TECHNOLOGY
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1.0 INTRODUCTION

The Center for Integration of Medicine and Innovative Technology (CIMIT) has completed its third year of DoD support. CIMIT is a non-profit consortium of world-leading academic and research institutions founded by Partners HealthCare System, Massachusetts General Hospital, Brigham and Women's Hospital, Massachusetts Institute of Technology, and the Charles Stark Draper Laboratory. The overall goal of the program has evolved beyond minimally invasive therapy to include other aspects of acute care using high technology approaches.

> ***CIMIT's mission is to improve patient care by bringing together scientists, engineers, and clinicians to catalyze development of innovative technology, emphasizing minimally invasive diagnosis and therapy.***

Coupling to DoD Needs

CIMIT Leadership has continued to stress the coupling of its activities to DoD needs, particularly in Combat Casualty Care. New and enhanced thrusts in simulation of medical procedures (for combat medic training) and acute care have been initiated and developed, as described below. The process of formal reviews, reports, and meeting participation has been supplemented with an exchange of leadership on-site meetings. Col Dean Calcagni, MD, Col Ron Poropatich, MD and Drs. Fred Pearce and Sean O'Donnell visited CIMIT for a two day informal briefing on 1-2 November, 2000. Subsequently, CIMIT Associate Directors James Muller, MD and Kirby Vosburgh, PhD made several separate trips to the Telemedicine and Advanced Technology Research Center (TATRC) and The US Army Medical Research and Materiel Command. CIMIT provided a highlight demonstration at the DoD Combat Casualty Care (ATACCC) Meeting in Ft. Walton Beach, Florida, on 10 September 2001.

Following the terrorist attacks on 11 September 2001, CIMIT Investigators and Leadership met to define enhancements of ongoing programs that might better serve national needs, and a preliminary outline of these efforts was submitted to the DoD for consideration.

Progress in Scientific Programs

During the past year, CIMIT has made significant progress in the Enabling Technologies and Clinical Challenges especially in the areas of Combat Casualty Care, Tissue Engineering and Vulnerable Plaque Detection and Treatment:

- The VIRGIL Chest Tube Insertion Simulator was developed to provide a near term deliverable from the CIMIT Simulation program. The system design and prototype has been completed and tested successfully. See Sections 1.2 and 2.5.
- A team at Massachusetts General Hospital led by Drs. Thomas Gill and David Zaleske demonstrated a new approach to repairing knee meniscus cartilage using tissue engineered materials in a large animal model system. See Section 2.4.
- A team from Draper Laboratory and Brigham and Women's Hospital has demonstrated the detection of immunoproteins using a micro-miniaturized sensor. See Section 2.4.
- The Vulnerable Plaque Program initiated a series of weekly seminars with world leaders addressing the development, analysis, and treatment of vulnerable plaques, which are believed to be a primary cause of arterial thrombosis. See Sections 1.2 and 3.2.

- A team of investigators from Draper Laboratory and Massachusetts General Hospital has achieved significant milestones in both fabrication and testing of microfabricated vascular scaffolds for tissue engineering. See sections 1.2 and 2.4.
- Several new research projects were begun this year, among them:
    - Drs. Adzik and Flake (Pennsylvania) are studying mesenchymal stem cells in tissue engineering,
    - Dr. MacLaughlin (MGH) is studying novel approaches for delivering proteins to disease sites to inhibit cancer growth,
    - Dr. Steven M. Greenberg, (MGH) received final approval from the HSRRB at the DoD and have begun to study vascular reactivity by functional MR in cerebral amyloid angiopathy, and
    - David Torchiana, MD (MGH) and Robert Howe, PhD (Harvard University) received final approval from the DoD for their animal protocol and have initiated studies to develop virtual fixtures for robot-assisted minimally invasive therapy.

1.1 Key Accomplishments by Program – Project – Principal Investigator

Endovascular Devices – Cardiomyocyte Repopulation – Steven Oesterle, MD, MGH

The major progress in the cardiomyocyte repopulation project this year was the initial preclinical evaluation of a catheter-based, transvascular method for intramyocardial delivery of an autologous adult stem cell/collagen biogel substrate. This represents the cumulative efforts of (1) catheter design, development, and refinement, (2) identification and development of methods for harvest and culture of an adult bone marrow progenitor cell population, and (3) initial experiences with cell/catheter compatible biogel delivery vehicles.

Minimally Invasive Surgery – Endoscopic Anastomosis – David Torchiana, MD, MGH

Chronic studies in a large animal model demonstrated that FocalSeal surgical sealant is an effective hemostatic adjunct without associated tissue toxicity when applied to blood vessel anastomoses sites. Also, four laboratory studies were completed using the new, updated Zeus Robotic Surgical System, and this system functioned properly in all cases.

Minimally Invasive Surgery - Craniofacial Surgery Planning – Leonard Kaban, MD, MGH

This past year the user interface been improved for six degree-of-freedom manipulation of 3-D graphical objects using a standard mouse. Also, an upgraded version of a 3-D axis manipulation tool was implemented in the current version of the 3-D Slicer.

Minimally Invasive Surgery - Robot-Assisted Surgery – Robert Howe, PhD, HMS

CT imaging protocol and image processing algorithms were developed to extract the artery location. A virtual fixture was successfully programmed on the Zeus robot system and demonstrated its effectiveness in laboratory trials.

Image Guided Therapy - Focused Ultrasound for Cancer Tx – Ferenc Jolesz, MD, BWH

During this past year, the phased array technology was translated to pre-clinical testing in an animal model. The team tested a 208 channel completely integrated system by sonicating rabbit tissues and implanted tumors. This system was completed based on the tests and is now ready for clinical trials.

Image Guided Therapy - Automated Image Segmentation – Carl-Frederik Westin, PhD, BWH

This year team continued to incorporate recent results in segmentation of medical data using geodesic active contours into the existing segmentation scheme from last year based on adaptive filtering. Active contours in 3D mean that a 2D surface is evolved iteratively in 3D. This work builds on the experience in the Surgical Planning Laboratory at Brigham and Women's Hospital, over the past decade, in segmenting clinical data for neurosurgical applications. Further, the team has been developing a user-steered segmentation algorithm based on the livewire paradigm. Livewire is an image feature driven method that finds an optimal path between user-selected image locations, thus reducing the need to manually define the complete boundary. The team has introduced a new image feature, based on local phase, which describes local edge symmetry independent of absolute gray value. The phase is a natural bi-product from the filters used in the adaptive filtering scheme presented last year in the project. Because phase is amplitude invariant, the measurements are robust with respect to smooth variations, such as bias field inhomogeneities present in all MR images. In order to enable validation of the phase-wire segmentation software, a system has been created that continuously records user interaction and automatically generates a database containing the number of user interactions, such as mouse events, and time stamps from various editing modules. The team has conducted validation trials and obtained expert opinions regarding its functionality.

Tissue Engineering - Degradable Conductive Polymers – Robert Langer, ScD, MIT

This year the CIMIT team proposed a novel approach to the creation of bioerodible polypyrrole (Ppy). In this novel paradigm the rate of erosion of Ppy is controlled by the hydrolysis and ionization of pendant groups followed by the solubilization of Ppy oligomers. The team has verified the hypothesis that solubilization of Ppy solid and thin film substrates, via ionizable side chain moieties, can occur under physiological conditions.

Tissue Engineering - Polymer-Based Gene Delivery Platforms – Robert Langer, ScD, MIT

This year the CIMIT team reports the first accelerated discovery approach for finding synthetic transfection vectors. This built on the team's previous work in synthesizing poly(□-amino ester)s. Importantly, initial results suggest several new polymers in the libraries generated have higher transfection efficiency than existing synthetic vectors in cell based assays.

Tissue Engineering - Trans-Dermal Drug Delivery – James Weaver, PhD, MIT

During the past year new approach to computer simulation of spatially complex systems was identified. The team has obtained very encouraging results for a simulation of the transport of potent agents through the skin due to exposure of a small amount of the compound to the surface of the skin.

Tissue Engineering - Synthesis of Vascularized Living Systems – Joseph Vacanti, MD, MGH

Significant milestones in both fabrication and testing of microfabricated vascular scaffolds have been reached during this year. Foremost among these is fabrication of the latest vascularized network design, TEP-2, in both silicon micromachined and polymer cast scaffold materials. Initial fluid dynamic testing of these devices indicates that the twin goals of reduced pressure losses and more uniform flow have both been accomplished. Polymer fabrication in both biocompatible and biodegradable matrices is moving ahead swiftly. Molds produced from PolyDiMethylSiloxane (PDMS), a biocompatible polymer, have been produced in both two- and three-dimensions. Three-dimensional vascular beds have been connected in parallel and run through initial fluid dynamic qualification studies. Bonded layers of biodegradable PLGA molded films have also been produced, a major milestone.

Tissue Engineering - Structures to Enable Vascularization – Jeffrey Borenstein, PhD, Draper

Major milestones in the design and microfabrication of scaffolds for endothelial cell seeding have been reached during the past year. In the area of design, several generations of networks have been produced, each representing a significant advance over previous designs. The first design with fully uniform flow, TEP-2, was generated on the wafer level and utilized to produce large numbers of silicon and polymer scaffolds for cell seeding. Design efforts were then transitioned to the modular networks TESTNET0 and TESTNET1, which are more suited to fluid dynamic experiments and biocompatibility studies. In a major advance, a new technique for generating mask layouts has been developed in which computational techniques are used to automatically produce vascular networks with desired flow characteristics. This new layout tool has been applied to the generation of photomasks for vascular designs, thereby reducing the cost of photomasks from $700 to $15 and the layout time required from 2 weeks to 1 day.

Tissue Engineering - Minimally Invasive Meniscal Repair – Thomas Gill, MD, MGH

Studies performed over the past year demonstrated that a cell-based therapeutic approach can be used in the articular environment in a large animal model of meniscal tears. Further investigation during the second year sought to define the best delivery material and the best pre-seeding conditions of the reparative cells onto candidate scaffolds. A clinically applicable approach combining this technique with arthroscopic surgery might be developed based on these studies.

Tissue Engineering – Treatments for Ovarian Cancer – David MacLaughlin, PhD, MGH

During the past year, resorbable polyglycolic acid biopolymer matrices impregnated with cells transfected with the MIS gene were successfully implanted in over 80 immuno-compromised mice and bioactive MIS produced and absorbed by the blood stream. The effect of different sized biopolymer implants on the resulting serum MIS concentrations was also determined.

Tissue Engineering - MSCs for Tissue Engineering – Scott Adzick, MD, UPENN

During the past year, the team made significant progress toward the clinical utilization of mesenchymal stem cells. Due to progress from other investigators in the field, it is clear that a mesenchymal stem cell of small phenotype, rather than the large fibroblastic phenotype used in our previous studies, has significantly greater differentiative capacity *in vitro*, and contains a higher frequency of CFU-f forming cells.

New Initiatives - Outcome Assessment in Menorrhagia – Johanna Bosch, PhD, MGH

Two different quality-of-life questionnaires have been composed. One questionnaire includes the standard version of the HUI (4 weeks) and the standard version of the EuroQol-5D ('your health today'). The second questionnaire includes the standard version of the HUI, but two slightly different versions of the EuroQol-5D.

Simulation – Procedural Simulation – Steven Dawson, MD, MGH

On September 25th, 2000, at the ATACCC 2000 meeting, the team offered to produce a prototype simulator which would demonstrate how the individual design elements of our larger research program could be unified into a working system. Our deliverable was a chest tube simulator at the 2001 ATACCC meeting, because effective training in insertion of a chest tube was a stated RAD II/Combat Casualty Care need. On September 10th, 2001, our group demonstrated the trainer to General Parker, General Kevin Kiley, Colonel James Kirkpatrick and Colonel Robert Vandre, among others, during the ATACCC meeting. The coincidence of our successful demonstration of a combat care training system which was designed to Special Forces

specifications on the night before the tragic events of September 11th continues to haunt the members of the simulation team.

Trauma and Critical Care – Microsensors – Christopher Dube, PhD, Draper

The key milestones of the past year include; Biological detection of the immunoprotein IgG using individually functionalized μCANARY element. Biological detection of an immunoprotein using individually functionalized μCANARY elements came as a result of the progress made during this same period in developing surface chemistry for antibody attachment to the sensor elements. The first biological detection of *Legionella pneumophila* antigen in urine using the 9-element μCANARY sensor. The 9-element μCANARY sensor was functionalized with four different antibodies (two polyclonal and two monoclonal) using BioDot addressing of individual sensor elements. The Legionella and IgG experiments described here represent the first time that individual elements of the μCANARY have been functionalized and demonstrated selective response. Correlation of the frequency shift of binding of biologically labeled particles with the optical density of the bound particles. This represents a major achievement in that it was an independent means of quantitation of the sensor frequency response to a biological target with an independent measure of binding (optical density) of the biological target to the sensor elements. The first reproducible means of regenerating the sensor surface for recycling of μCANARY sensors. Finally, Draper Laboratory began funding Prof. David Kaplan at Tufts University to develop alternative affinity ligand reagents (ALRs) for the detection of microorganisms, based on peptides expressed on the outer surface coat of phage. These ALRs will be used in conjunction with the μCANARY sensors for detection of microbial pathogens.

Trauma and Critical Care – Hematoma Detection – Geoffrey Ling, MD, PhD, USUHS

This past year, the team reported success in completing study of the RAFTS as it is applied to diagnosis of intracranial hematomas, pneumothorax and compartment syndrome. In brief, the team reported the findings of *in vitro* testing using cadaveric pig brains. The results from this work demonstrate that the RAFTS can differentiate hematomas from brain and skull. Subsequently, the team reported the completed *in vivo* study that was performed in live anesthetized pigs. These studies show that the RAFTS can accurately detect the presence of hematomas at epidural, intraventricular, subdural and intraparenchymal sites in a clinically relevant model. Also in pigs, RAFTS can also detect as little as a 10% pneumothorax and as little as 2cc of either blood or saline in the muscle compartment

Trauma and Critical Care – Assessment of Ischemia – Juan Carlos Puyana, MD, BWH

This past year, the team tested the concept that the animals breathing spontaneously may be capable of compensating the elevation of tissue PCO_2 by reaching high respiratory rates and induced compensatory hyperventilation. To test this hypothesis the team used a rat model of uncompensated shock. A multiparameter (PO_2, PCO_2, pH) monitoring fiberoptic catheter was used for the assessment of tissue perfusion during shock.

Vulnerable Plaque – VP Detection and Treatment – James E. Muller, MD, MGH

During the past year the Vulnerable Plaque Program launched its weekly seminar series. The program consisted of a weekly series of topics on various aspects of vulnerable plaque given by investigators within and outside the Boston community. Also, Dr. James Muller along with Drs. Peter Libby and Valentin Fuster created the First International Symposium on Vulnerable Plaque. The symposium will be held in Cambridge on October 4 – 5, 2001 at the MIT University Park Hotel. Dr. Muller was able to recruit key investigators from around the world.

Vulnerable Plaque – OCT for Vulnerable Plaque Detection – Brett Bouma, PhD, MGH

This past year the team has advanced the capabilities of OCT for imaging *in vivo* by resolving three key technical issues. First, the team has developed methods for displacing blood from the iliac and aorta using balloon occlusion and saline flush. Second, the team has demonstrated a sufficient image acquisition rate to avoid motion artifact due to respiration and pulsatile blood flow. Finally, the team has demonstrated that characteristic features in plaques can be resolved using a catheter that provides a resolution of approximately 10 microns.

Stroke - Recanalization for Acute Stroke – Ramon Gilberto Gonzalez, MD, PhD, MGH

This past year four animals were studied with the bypass setup: in two animals the correct flow rate was achieved and brain tissue in the lesion area was maintained viable for 60-90 minutes, while in one animal the flow rate was too low and the lesion tissue infarcted, while in one animal the flow rate was too high and hemorrhage resulted. These results identify the future directions for this part of the project: to improve flow stability using a better blood pump and to improve the means of obtaining the correct flow rate in each animal by measuring or calculating accurately the fluid pressure at the tip of the microcatheter within the brain.

Stroke - Optical Monitoring of Stroke – Walter Koroshetz, MD, MGH

This year the CIMIT team fabricated a Continuous-Wave Imaging System with enough lasers and detectors to image stroke related effects over the entire brain surface. Also, the team developed accurate modeling of photon migration through the complex structure of the human head by using Monte Carlo techniques to simulate the propagation of photons through tissue and obtain a spatial sensitivity.

Stroke - Functional MRI in Cerebral Amyloid Angiopathy – Steven Greenberg, MD, MGH

This year, four control patients were studied using fMRI. Data analysis revealed a robust response in blood flow to both visual stimulation and CO_2 inhalation.

Technology Assessment - Inpatient Costs in Stroke – G. Scott Gazelle, MD, PhD, MGH

This past year, the CIMIT team's principal research efforts have continued to focus in three principal areas: Stroke, Vulnerable Plaque, and the Operating Room of the Future Project.

Core Programs – Regulatory Affairs – John Smith, MD, JD, MGH

This year the CIMIT Regulatory Affairs team assisted the FDA with in implementing the "least burdensome means" concept as required by the Food and Drug Administration Modernization Act of 1997 (FDAMA). The Regulatory Affairs Program has functioned as a point of access to FDA's Center for Device and Radiological Health, providing information and academic resources to FDA managers and line reviewers.

1.2 Highlight Projects

VIRGIL Chest Trauma Training System

Principle Investigator: Steven L. Dawson, MD MGH

On September 25th, 2000, at the ATACCC 2000 meeting, the CIMIT Simulation Team led by Steven L. Dawson, MD (MGH), offered to produce a prototype simulator which would demonstrate how the individual design elements of CIMIT's larger research program could be unified into a working system. **Because effective training in insertion of a chest tube was a**

stated RAD II/Combat Casualty Care need, CIMIT's deliverable was a chest tube simulator at the 2001 ATACCC meeting. On September 10th, 2001, our group demonstrated the trainer to General Parker, General Kevin Kiley, Colonel James Kirkpatrick and Colonel Robert Vandre, among others, during the ATACCC meeting. The coincidence of our successful demonstration of a combat care training system which was designed to Special Forces specifications on the night before the tragic events of September 11, 2001 continues to haunt the members of the CIMIT Simulation Team.

The VIRGIL chest trauma training system (Figure 1) incorporates haptics, tissue-tool interactions, real-time graphics and augmented reality to present a realistic experience of assessing and treating penetrating trauma in a simulated battlefield scenario. A free-standing but integral web-based and CD-compatible educational curriculum accompanies the training system, presenting treatment doctrine based upon the standards expressed in Tactical Medicine in Naval Special Warfare, Tactical Management of Urban Warfare Casualties in Special Operations, the DMRTI C4 Handbook, AMEDD handbooks, and the 1988 NATO Manual. The entire system is portable and can be run on standard 110 volt AC power or as a free-standing unit for field training using an integral 12 volt DC power source. **See Section 2.5 for full report.**

Figure 1. Dr. Steven L. Dawson, MD (right), team leader of the CIMIT Simulation Program, describes the **VIRGIL Chest Trauma Training System** to MG John S. Parker, Commanding General, USAMRMC.

Synthesis of Vascularized Living Systems on a Silicon Platform
Principal Investigator: Joseph Vacanti, MD, MGH

This project seeks to develop tissue-engineered devices composed of living cells on matrices which, upon implantation, are vascularized either *in vitro* or *in vivo*. The primary approach is to synthesize vascularized systems from the platform of 2-D silicon microfabrication technologies with adaptation to support three-dimensional living devices.

Significant milestones in both fabrication and testing of microfabricated vascular scaffolds have been reached this past year. Foremost among these is fabrication of the latest vascularized network design, TEP-2, in both silicon micromachined and polymer cast scaffold materials. Initial fluid dynamic testing of these devices indicates that the twin goals of reduced pressure losses and more uniform flow have both been accomplished. Polymer fabrication in both biocompatible and biodegradable matrices is moving ahead swiftly. Molds produced from PolyDiMethylSiloxane (PDMS), a biocompatible polymer, have been produced in both two- and three-dimensions. Three-dimensional vascular beds have been connected in parallel and run through initial fluid dynamic qualification studies. Bonded layers of biodegradable PLGA molded films have also been produced, a major milestone.

Also, significant progress in the speed, reliability, and reproducibility of fabricating microfluidic devices has been made this past year. High-resolution photomasks were replaced by transparency photomasking for microfluidic applications allowing rapid prototyping, wherein a single researcher can design, print, micropattern a silicon mold, and create a new set of polymer devices within 1 day. In addition, once silicon molds are fabricated, they can be reused for casting indefinitely, requiring only 2 hours to build subsequent devices. Efforts to clamp etched wafers and flat substrates that proved unreliable in the past were replaced by plasma-assisted bonding of PDMS films to Pyrex wafers, significantly improving the reliability of sealed microfluidic devices and enabling reproducible cell culture without leaks or contamination (Figure 2). These processes were extended from two-dimensional microfluidic networks to interconnected three-dimensional networks using layer stacking and alignment (Figure 3). The processes together make up a robust tool-set for designing, fabricating, and testing cell culture in three-dimensional microfluidic scaffolds. **See Section 2.4 for full report.**

Figure 2 - *Topdown image of plasma-assisted bonding of PDMS films to fabricate a microfluidic network.*

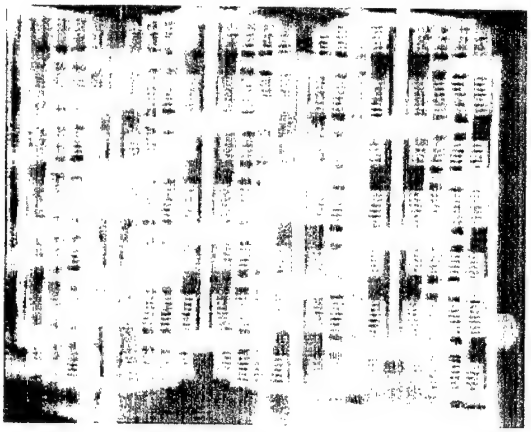


Figure 3 – *Top down image of multilayer bonded PDMS microfluidic network perfused with fluorescent dye.*

Detection of Vulnerable Plaque using Optical Coherence Tomography
Principal Investigator: Brett Bouma, PhD, MGH

The primary goal of this year's research was to apply optical coherence tomography (OCT) for monitoring plaque development and response to systemic therapy in an animal model for atherosclerosis. Prior to this work, no longitudinal studies have been conducted within individual animals. The advantage of OCT is that cross-sectional images with a resolution approaching that of histopathology can be obtained *in vivo* (without sacrifice) so that disease can be monitored over time in individual animals. In order to make longitudinal studies with OCT practical, several technical issues have been addressed including imaging in the presence of blood, avoiding motion artifacts and providing sufficient resolution for plaque characterization.

A second goal of the research was to investigate the ability of OCT to characterize plaque composition. As there is no existing gold-standard for plaque characterization *in vivo*, the team has conducted an extensive *in vitro* study.

The team has advanced the capabilities of OCT for imaging *in vivo* by resolving three key technical issues. First, the team has developed methods for displacing blood from the iliac and aorta using balloon occlusion and saline flush. Second, the team demonstrated a sufficient image acquisition rate to avoid motion artifact due to respiration and pulsatile blood flow. Finally, the team demonstrated that characteristic features in plaques can be resolved using a catheter that provides a resolution of approximately 10 microns (Figure 4).

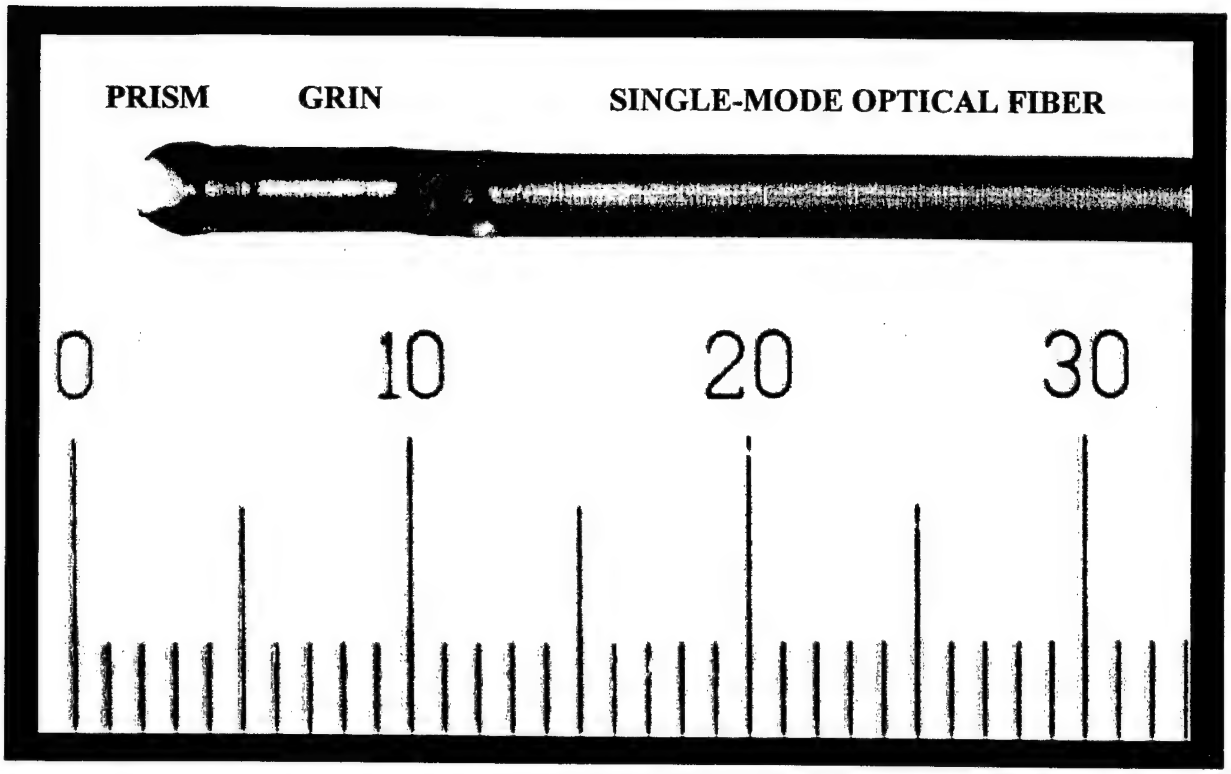


Figure 4. Inner core (250 μm diameter) of the new 1.4 F OCT catheter. In this Figure, the outer sheath of the catheter was removed for improved visualization of the distal optics.

The CIMIT team has demonstrated that OCT imaging of the rabbit iliac and distal aorta can be performed in live animals without sacrifice. The images have demonstrated that OCT provides adequate resolution and contrast to identify focal lipid rich plaques and that the extent of disease can be quantified (Figure 5). Consistent with the findings of the *in vitro* study, lipid pools within the aorta wall present as signal poor regions (arrows in the Figure). In Figure 2B, a thin fibrous cap is seen as a layer of high signal overlying the lipid pool and has a thickness of approximately 20 μm. **See Section 3.2 for full report.**

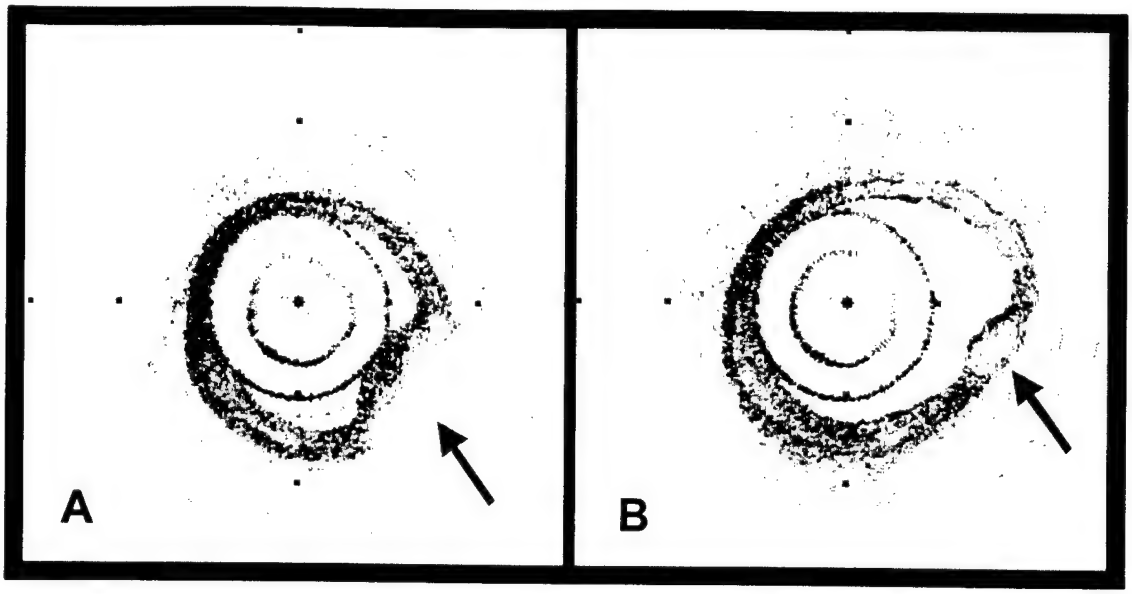


Figure 5. OCT images of aorta of Watanabe rabbit following 2 months high cholesterol diet. Low signal areas with dark overlying layer (arrows) denote lipid pools with fibrous caps.

1.3 Highlight Organizational Accomplishments

- In August 2001, CIMIT moved to the new Partners Research Building at 65 Landsdowne Street, in Cambridge, MA. The new facilities provide state-of-the-art offices, research areas and connectivity, with more space than was previously available at CIMIT's offices in Boston,
    - This new facility also provides a central location for the major research/program areas of Procedural Simulation, Medical Simulation and the Advanced Procedure Room and Innovation Laboratory (APRIL). This accomplishment is the realization of the original goal and focus of CIMIT-- to co-locate these activities and staff them with clinicians, engineers, students, fellows, etc.,
- CIMIT Administrative staff completed a one-day visit to TATRC, Ft. Detrick, MD to discuss improvements in administrative processes. This was a successful and pleasant visit,
- The Annual Report for the Second year of CIMIT Operations was submitted in revised form at the request of the DoD COR, and accepted,
- The Fourth Year proposal for CIMIT was submitted to DoD,
- Additional content has been written for the CIMIT external website, at www.cimit.org, Utilizing the power of the internet and database technologies, the CIMIT website has proven to be a useful and engaging tool not just for general information seekers, but for those stakeholders who want to actively participate. CIMIT has developed a web-based knowledge management system for members--called "myCIMIT". This site archives CIMIT FORUMs and provides project management capabilities and meeting summaries and a quick reference for CIMIT contacts,
- The project financial system was converted to the federal fiscal year to simplify budgeting and reporting,

- The plan for FY 2002 was completed with the approval of 37 new or continuing programs. On a cost basis, roughly one third of this work is comprised of new activities,
- CIMIT Industry Collaborations with 25 companies provided contributions totaling $6.3 million (fee, in-kind contributions, and sponsored research) through various collaboration opportunities: Industry Liaison Program, Vulnerable Plaque Program, OR of the Future and Endovascular Device Lab,
- CIMIT has designed a communications program to address its diverse communication needs, audiences and resources. This includes published reports, CDs of conferences and presentations at National and local CIMIT Forums, and
- The CIMIT Office of Technology Development
    - assisted in securing $4.6 million in sponsored research over the next five years to continue advanced development of CIMIT-supported programs,
    - worked with the consortium licensing offices to register over sixty new invention disclosures related to CIMIT-supported research,
    - designed, produced and distributed individual customized Investigator Handbooks for each of the CIMIT Principal Investigators to assist in their Regulatory Approval processes, Research Management programs, and the Intellectual Property management systems, and
    - supported the formation of two new ventures resulting from CIMIT-funded programs.

National and International CIMIT

CIMIT continues to develop a national and international network. CIMIT enables researchers to explore, devise and implement new technologies, while providing real breakthroughs in research through the collaboration of academic medical centers, industry and the military. The following highlight our national and international activities to date:

- Continued collaboration in medical simulation "Enabling Technologies for Medical Simulation" with TATRC,
- Established a third year of research funding at Harvey Mudd College to develop biosensors,
- Continued collaborative research program in telemedicine "Remote Stroke Videoconferencing Project (RSVP): Telemedicine-Enabled Remote Diagnosis and Therapy" with Partners HealthCare System; and the plan is to migrate this program to a national scale,
- Continued funding of the microwave research project "Application of Microwave Imaging to Rapid Non-Invasive Detection of Intracranial Hematoma" at USHUS, and
- In April, 2001 the premier tertiary hospital system in Saudi Arabia became the newest member of the CIMIT consortium and the first international academic member. The King Faisal Specialist Hospital and Research Center (KFSH) entered into a five-year agreement with CIMIT as a means of strengthening their research program and gaining access to novel and cutting-edge technologies that will improve patient care.

2.0 ENABLING TECHNOLOGIES

2.1 ENDOVASCULAR DEVICES

Task 1: Cardiomyocyte Repopulation using Percutaneous Delivery of Tissue Engineered Systems

Principal Investigator: Steven Oesterle, MD, Massachusetts General Hospital (MGH), Boston, MA

Over 1.5 million adults experience acute myocardial infarction each year. More than 500,000 die from the event. Many others survive with significant impairment of left ventricular function. A multitude of diseases unrelated to atherosclerosis, can also result in varying degrees of heart failure-sustained hypertension, viral myocarditis, and valvular insufficiency frequently lead to intractable ventricular dysfunction. Congestive heart failure (CHF) is amongst the most frequent diagnoses for patients admitted to acute care hospitals. CHF is associated with the longest lengths of stay for any of the cardiac Diagnostic Related Groups (DRGs). Acute and chronic care of patients with heart failure consumes billions of health care dollars. Beyond diuretics and other pharmaceutical preparations that "unload" failing hearts are few effective treatments for advanced heart failure. Adult mammalian myocardial cells are generally believed to be terminally differentiated with little or no capacity for repair or proliferation. Unlike skeletal muscle, irreversible injury to myocardial tissue predictably leads to akinesis, fibrosis and thinning.

The purpose of this project is to develop a tissue engineered device to replace myocardial muscle damaged by ischemia or inflammation and to develop a percutaneous catheter system for delivery to the heart using an *in vivo* porcine model.

The focus of cell source for cellular cardiomyoplasty shifted this year from fetal cardiomyocytes to mesenchymal stem cells (MSC). Recent data suggest that MSCs have the potential to differentiate into cardiomyocyte-like cells.

MSCs have the advantage of being easily harvested and expanded from autologous bone marrow, thus avoiding issues such as rejection, need for immunosuppressive therapy (and its subsequent complications), and ethical issues referable to fetal/embryonic research and medical application. During this year, harvest, maintenance, and GFP-labeling protocols for this cell line have been developed.

The current "delivery vehicle" is a collagen/GFP labeled, mesenchymal stem cell (MSC) biogel with viscous properties compatible with the percutaneous catheter delivery system. This cell-biogel preparation is easily injected through (and not damaged by) the catheter system and forms three-dimensional architecture when it reaches room or body temperature. The MSCs maintain viability and proliferative properties within this matrix.

Key Results: The major progress in the cardiomyocyte repopulation project this year was the initial preclinical evaluation of a catheter-based, transvascular method for intramyocardial delivery of an autologous adult stem cell/collagen biogel substrate. This represents the cumulative efforts of (1) catheter design, development, and refinement, (2) identification and development of methods for harvest and culture of an adult bone marrow progenitor cell population, and (3) initial experiences with cell/catheter compatible biogel delivery vehicles.

Specific Aim 1: To refine percutaneous catheter devices to achieve quantitative myocardial cell transfer.

Progress: The focus of cell source for cellular cardiomyoplasty shifted this year from fetal cardiomyocytes to mesenchymal stem cells (MSC). Recent data suggest that MSCs have the potential to differentiate into cardiomyocyte-like cells.

MSCs have the advantage of being easily harvested and expanded from autologous bone marrow, thus avoiding issues such as rejection, need for immunosuppressive therapy (and its subsequent complications), and ethical issues referable to fetal/embryonic research and medical application. During this year, methods to harvest and maintain porcine bone marrow MSCs were developed.

In addition, optimal protocols were developed to label these cells with Green Fluorescence Protein via genetic viral transduction with vesiculostomatitis virus. This 'labeling" provides an identification method to distinguish donor-host relationships post cellular transplantation

A series of *in vitro* experiments were performed to characterize the material / cell interaction with the plastic sleeve that lines the extendable nitinol needle of the cell transfer catheter. Mesenchymal stem cells (MSC) did not have shear-related trauma or induced apoptosis when injected through this catheter system. MSC viability and proliferation *in vitro* does not differ between catheter injected and control populations.

A Materials Transfer Agreement was finalized which facilitated receipt and experimentation with the modified catheters from TransVascular, Inc (Menlo Park, CA).

Initial Preclinical Investigation
After having developed methods to harvest, genetically label, maintain, multiply, and recombine bone marrow MSCs in a compatible hydrogel "delivery vehicle" (Specific Aim 2), the proposed percutaneous, transvascular, .intramyocardial approach for cardiac cell delivery was tested in 6 Yorkshire swine (50-60kg).

Bone marrow was harvested from each pig and marrow stromal cells were selected, genomically transduced with green fluorescence protein, expanded in culture, and resuspended in collagen hydrogel (Figure 1) marked with tissue dye. Coronary venous access was secured a specialized catheter system, and transvenous myocardial punctures were performed through the anterior intraventricular coronary vein (Figure 2) with a composite catheter system (TransAccess^{TM}) (Figure 3) incorporating a phased-array ultrasound (Figure 4) tip and sheathed, extendable nitinol needle. A microinfusion catheter was advanced through the needle deep into remote myocardium and "rows" of injections were performed in the anterior, lateral, septal, and apical ventricular walls (Figure 5). Animals were sacrificed at time 0(N=2), time 14(N=1,+1 control/collagen biogel only), and 28 days (N=2), and hearts examined.

No death, cardiac tamponade, ventricular arrhythmia or procedural complications occurred. Gross inspection demonstrated no evidence of myocardial perforation, and biogel/black tissue dye was well localized to sites corresponding to procedural fluoroscopic landmarks (Figure 6).

Microscopic analysis demonstrated needle and microcatheter tracts, biogel delivery, and positive cell-shaped structures under direct fluorescence (Figure 7). Immuno-histochemical evaluation of this autologous, "donor" bone marrow cell population is currently being investigated.

Figure 1- Porcine mesenchymal stem cells genetically transduced with green fluorescence protein, resuspended in collagen hydrogel. Direct green immunofluorescence, 20X magnification.

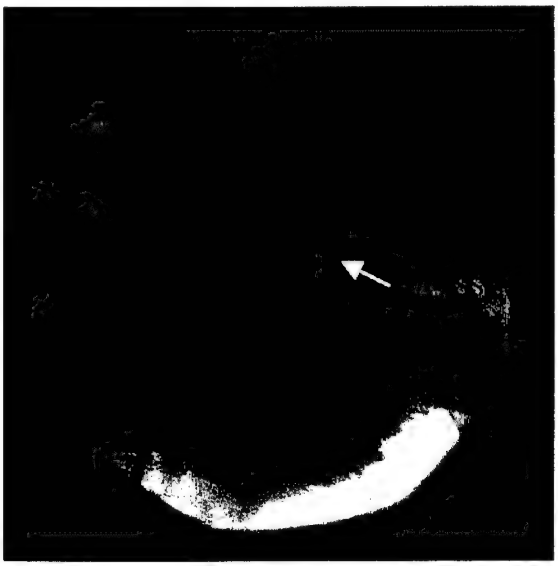


Figure 2 – Percutaneous Transvenous intramyocardial needle puncture – fluoroscopic image of catheter system engaging the coronary venous system. White arrow marks extended needle from the TransAccess catheter. CS = coronary sinus guiding catheter, SS = subselective catheter.

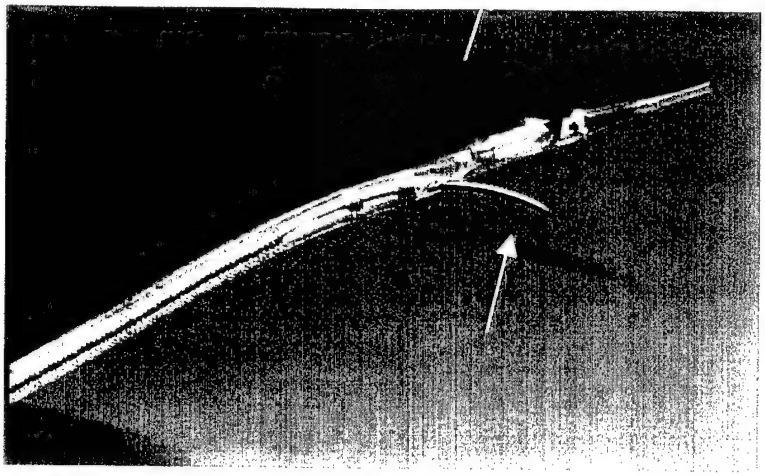


Figure 3 – TransAccess™ catheter –is able to navigate through coronary venous guiding catheters over a steerable guidewire. This composite catheter incorporates intravascular imaging at the distal tip (black arrow) and an extendable nitinol needle (white arrow)for transvascular,

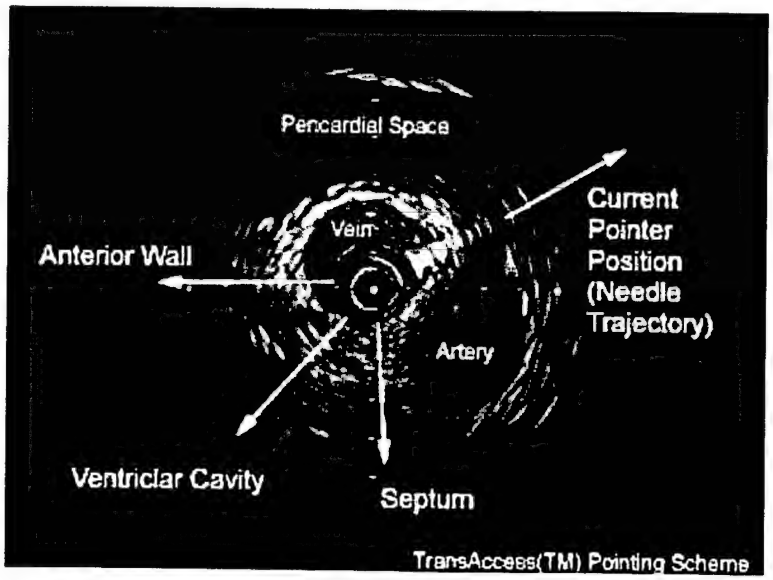


Figure 4 – Online intravascular imaging safely guides transvacular puncture and intramyocardial access.

Figure 5 – MicroLume™ microinfusion catheter (arrow) extended from TransAccess™ needle

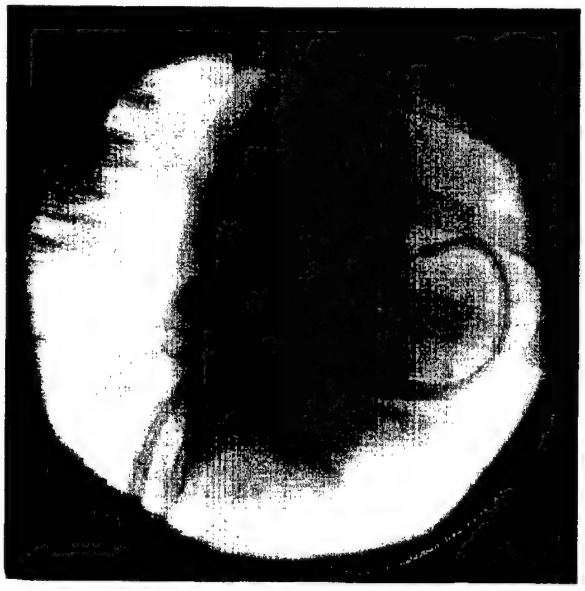


Figure 6 – Intramyocardial contrast injection demonstrating method for laying "rows" of tissue-engineered stem cell-hydrogel substrate (arrow)

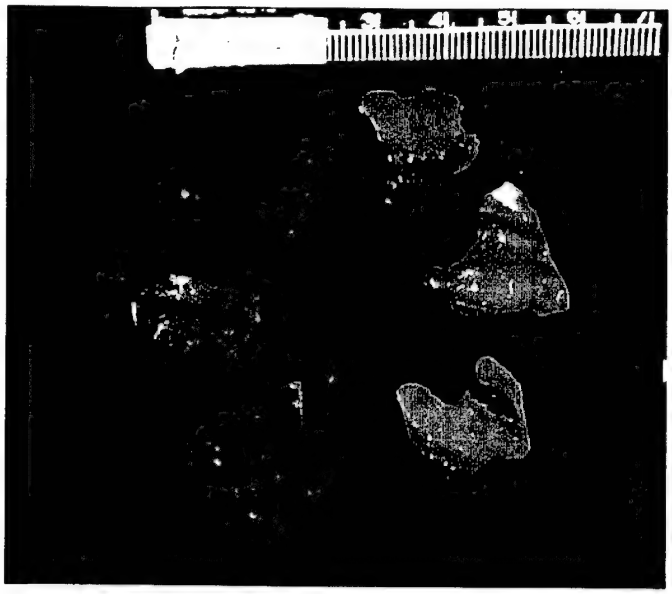


Figure 7 – Gross pathologic appearance of porcine myocardial tissue after cell-biogel transcatheter injection. Rows of the tissue engineered substrate are demarcated by black tissue dye.

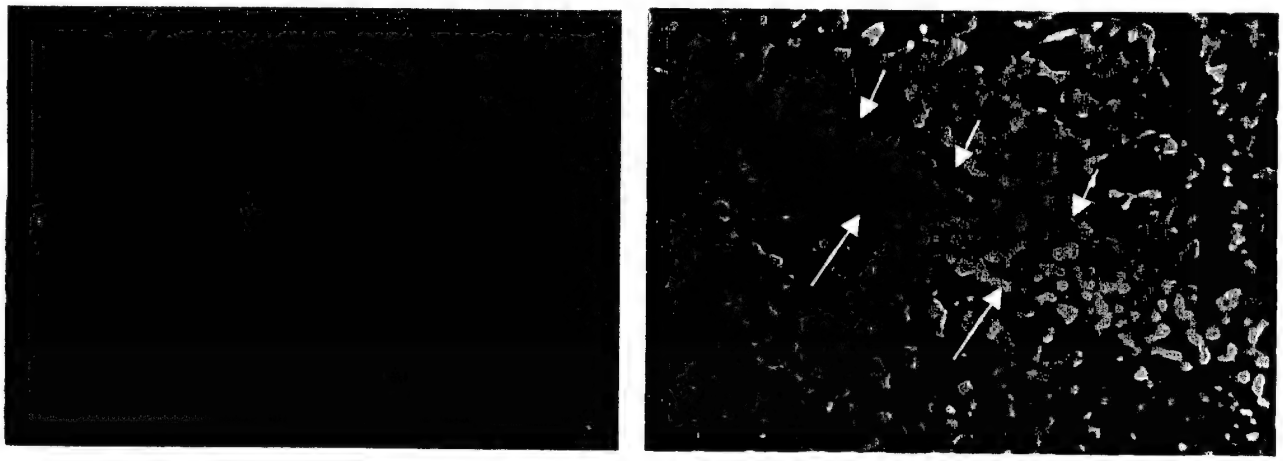


Figure 8 – Histologic section of needle tract (arrows) and mesenchymal stem cells identified by direct immunofluorescence. (20X magnification)

Plan: The emphasis this academic year will be to quantitatively evaluate this catheter based myocardial tissue engineering system in a porcine model of myocardial infarction. The stem cell population will be genetically labeled in a manner that will allow for cell quantification by non-invasive, positron emission tomography (PET) scanning. This PET scanning will provide valuable information on *in vivo* cellular proliferation potential, and improvements in regional viability, blood flow, and function.

Specific Aim 2: To develop a tissue engineered system of cardiomyocytes in hydro gel polymer for delivery into both healthy and damaged ventricles.

Progress: A series of *in vitro* experiments have demonstrated superior viability and proliferation capacity of MSCs within collagen hydrogels, compared to alginate and Pluronic 127 hydrogels. The collagen/MSC hydrogel preparation has been adapted to optimize viscosity profile needed for the percutaneous delivery method. Experiments are ongoing evaluating a variety of mixing protocols to improve MSC viability and proliferation within the alginate, matrigel, and Pluronic compounds.

Plan: The collagen/MSC hydrogel will be incorporated into ongoing mouse cell injection protocols and will be the delivery vehicle of choice for the percutaneous delivery system. The team will continue to attempt to optimize conditions to facilitate cell viability in, and usage of, alginate, matrigel, and Pluronic 127 biogels in cell transplant protocols by systematically adjusting external polymer attachment sequences (RGD, vitronectin, laminin).

Specific Aim 3: To evaluate applicability of adult and fetal cardiomyocytes and stem cells as catheter-delivered donors. To devise proper mixtures of hydro gel polymers, and growth factors to support cardiac cells for catheter delivery.

Progress: Ongoing experimentation includes direct cell injection into a mouse model:

1. Intra-myocardial cell delivery by direct injection into normal and injured hearts
2. Retrograde cell delivery through the venous system following cannulation and blockage of the coronary sinus

SCID-mice are anesthetized following a standard protocol of pre-anesthetic sedation. The mice are intubated and maintained on positive pressure ventilation during the procedure. A lateral thoracotomy in the left 4^{th} intercostal space is performed. The pericardium is opened and the LAD, and the coronary sinus is identified. The distal LAD is ligated with a 6-0 Silk suture. Through a 31G needle, 10^6 to 10^9 B-gal labeled cells are injected directly into the infarcted area or retrograde through the venous system via the coronary sinus. Our laboratory has recently developed a highly efficient technique for creating these so-called reporter cells *[Nasseri BA, Afting M, Pomerantseva I, Lien JG, Vacanti JP. Highly efficient stable retroviral gene transfer into primary endothelial cells for tissue engineering. 3rd Biennial Tissue Engineering Society Meeting, Orlando, USA 30.11. –3.12.2000].*

Mice are sacrificed at day 4, 7, 14 and 28. The hearts are removed, rinsed in water and frozen in O.C.T. Cryocuts are performed and slides are examined for GFP. Cells are counted, and immunohistology performed, to evaluate CM-differentiation of the mesenchymal bone marrow stem cells.

SCID-mice are anesthetized following a standard protocol of pre-anesthetic sedation. The mice are intubated and maintained on positive pressure ventilation during the procedure. Syngenic murine cells (from wild-type and transgenic mice) are placed in hydrogels and injected through a 31G needle directly into the left ventricular wall or retrograde through the venous system to the coronary sinus, in both normal and injured hearts. Injuries of the left ventricular wall are performed by ligation of the distal LAD with a 6-0 Silk suture. The cells are *β-gal*-labeled to facilitate identification of the transplanted cells after injection. The team used transgenic mice (FVB alpha MHC-*β-gal* or B6 129Srosa or MLC-IGF-1/alpha MHC-*β-gal)* as donors..

Mesenchymal bone marrow stem cells, mouse embryonic stem cells, skeletal myoblasts and fetal CM are harvested from these mice. Cells are mixed with hydrogels (Matrigel, Alginate, Collagen and Pluronic F-127) and injected into normal and injured hearts, as described. Mice are sacrificed and the hearts are prepared as previously described.

Plan: This experiment of comparison of cell delivery methods is ongoing. It is anticipated that this area of experimentation will provide insights into optimal delivery methods and mechanisms of cell transfer and engraftment.

Improved methods for hematopoietic and mesenchymal stem cell purification using flow cytometry (FACS) sorting will be investigated. The goal is to provide a "purer" stem cell source, with a higher effective working population. This method would facilitate a more clinically relevant approach in that cells could be transplanted shortly after the time of harvest, rather than having to wait for cellular *ex vivo* expansion.

2.2 MINIMALLY INVASIVE SURGERY

Task 1: Minimally Invasive Cardiac Surgery – Endoscopic Coronary Anastomosis

Principal Investigator: David Torchiana, MD and Jennifer White, MD, MGH

This project entails laboratory development of a robotic interface in cardiac surgery to ensure safe and effective clinical application of the technology. Since the project's initiation in 1999, endoscopic coronary artery bypass ("E-CABG") using the robotic interface ("Zeus", Computer Motion, Inc.) has been performed in sixty-eight laboratory animals. Advancements have been made in the anatomical positioning of instrument ports, internal mammary artery harvesting, and surgical skill in performing a non-beating heart anastomosis using the robotic interface. An upgraded Zeus robotic interface acquired in December 2000, reduced surgical case interruption due to computer errors. Novel instruments including a proximal anastomotic device ("Symmetry", St. Jude Medical, Inc.) and surgical clips ("U-Clip", Coalescent Surgical, Inc.) have been integrated into the procedure.

In the course of this work, it has been appreciated that endoscopic robot-assisted dissection of the internal mammary artery is time consuming and difficult. In humans the vessel tends to course deep in the transversus thoracic muscle and out of the thorascopic view of the surgeon. In an attempt to overcome this problem, manipulation of the robotic interface through CT-image guidance has been developed to assist in the video-endoscopic dissection of the internal mammary artery. Professor Howe's group from Harvard's Department of Engineering, has undertaken a joint effort with our laboratory to augment the internal mammary artery takedown procedure using computer-assisted computerized tomography (CT) guidance with 3-dimensional surgical instrument registration. The procedure could lead to safer clinical LIMA dissection, since the intra-operative movement of the robotic interface would be linked directly to the anatomical course of the vessel as "mapped out" by the patient's pre-operative CT scan. In addition, it expected that successfully linking data obtained from these pre-operative images to the procedure will enable instrument port placement to be optimized for each individual. This new emphasis on pre-operative image guidance should reduce instrument mechanical conflicts and increase the freedom of the instrument's movements to perform the task at hand.

Key Results: Chronic studies in a large animal model demonstrated that FocalSeal surgical sealant is an effective hemostatic adjunct without associated tissue toxicity when applied to blood vessel anastomoses sites. Also, four laboratory studies were completed using the new, updated Zeus Robotic Surgical System, and this system functioned properly in all cases.

Specific Aim 1: Characterization of FocalSeal surgical sealant as a hemostatic adjunct.

Progress: Project completed. See Quarterly Progress Report for the period January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report for the period January 1, 2001 through March 31, 2001.

Specific Aim 2: Perform acute and chronic evaluation of a new micro-anastomotic device on coronary arteries.

Progress: This past year, four cases using the proximal anastomosis device ("Symmetry", St. Jude Medical, Inc.) was successfully utilized, once endoscopically, in a large animal model to connect a saphenous vein to the ascending aorta, see Figures 1 – 3..

Plan: To continue to use the device being developed by St. Jude, Inc. to perform proximal graft anastomoses under fluoroscopic guidance.

Specific Aim 3: Development of a method for video-endoscopic coronary anastomosis.

This specific aim is to develop the closed chest E-CABG procedure in the laboratory during the pre-clinical investigation and move into the clinical setting in a four-step process. Laboratory studies directed towards device development and training of the surgical team will continue throughout all four phases. The clinical application will progress over a two-year period with phases of increasing surgical complexity and technical difficulty. Laboratory studies directed towards device development and training of the surgical team will continue throughout all phases.

Progress:

Laboratory

Since the project's initiation, the principal investigator/cardiac surgeon performed endoscopic coronary artery bypass procedures in sixty-eight canines using the Zeus Robotic Surgical System and HeartPort cardiopulmonary bypass techniques. Repeatedly, the left internal mammary artery (LIMA) and right internal mammary artery (RIMA) was harvested using the Zeus system in a completely endoscopic fashion. The enhanced system acquired in December permits the application of a broader spectrum of instrument designs (e.g. articulated instrument tips with ergonomic thumb and wrist control), as they become increasingly available. Opening of the pericardium and incising of the coronary artery was performed using the Zeus system with an articulating scalpel in the completely close-chested procedures.

Self-closing Nitinol sutures ("U-clips", Coalescent Surgical, Inc.) continued to be utilized to perform distal coronary anastomoses, joining vessels in an interrupted fashion in about the same time as it would have taken to perform a continuous distal anastomosis.

Plan:

Laboratory

The CIMIT team will continue to perform E-CABG procedures in the laboratory in timely preparation for upcoming clinical cases. The recent availability and FDA approval of a proximal anastomotic device will allow a series of closed chest multi-vessel bypass procedures in the laboratory which will be a major milestone in procedure development. The lab will serve as a site for investigating methods and instrumentation to support minimally invasive cardiac surgery while providing a training ground for specialized surgical skills.

Pre-operative computerized tomography image-guidance is to be investigated as a means of assisting internal mammary artery (IMA) harvesting and instrument port placement. The study endpoints include measurement of elapsed time for IMA takedown, accuracy (average thickness of the dissected pedicle containing the artery), error rates (injuries to the artery) in the dissection procedure, and surgical instrument's degrees of freedom to perform the procedure. It is expected that image guidance will hasten IMA dissection, while improving accuracy and decreasing injury

to the vessel. The endpoint of successful multivessel bypass patency will be assessed angiographically.

Clinical

The MGH team is one of two clinical sites in what will eventually be a five center clinical trial of IMA takedown with plans to enroll 250 patients in all. The DoD human use approval process is underway for this project. Dr. Torchiana is the Principal Investigator for this trial.

Illustrations: Minimally Invasive Cardiac Surgery – Endoscopic Coronary Anastomosis

These illustrations demonstrate the sequential use of proximal (Figures 1 - 3) and distal (Figures 4 - 7) anastomotic devices in this laboratory as discussed in the DoD report above.

Figure 1. Video-endoscopic creation of a proximal orifice in the canine ascending aorta using a 5.0-5.5 mm St. Jude Medical proximal aortic cutter.

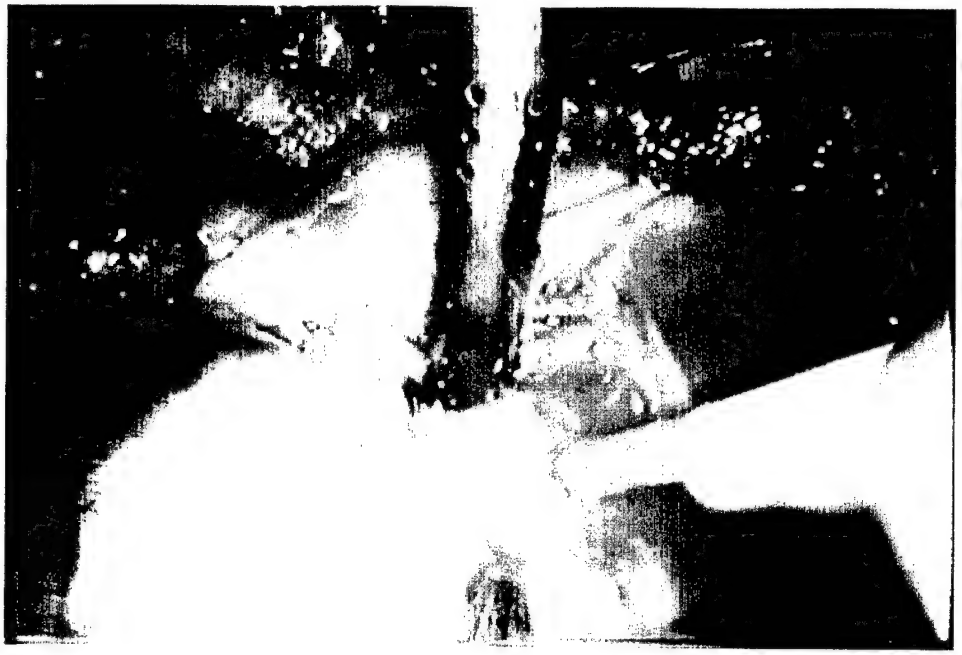


Figure 2. Deployment of the anastomotic device ("Symmetry", St. Jude Medical Inc., Minneapolis, MN) into the ascending aorta using video-endoscopic visualization of the surgical field.

Figure 3. Successful proximal anastomosis of saphenous vein to ascending aorta with the St. Jude anastomotic device in place.

Figure 4. Zeus robotic interface (Computer Motion, Inc, Santa Barbara, CA) effector instrument passing curved needle attached to nitinol anastomotic clip (Coalescent Surgical, Sunnyvale, CA) through the distal anastomotic site on canine heart.

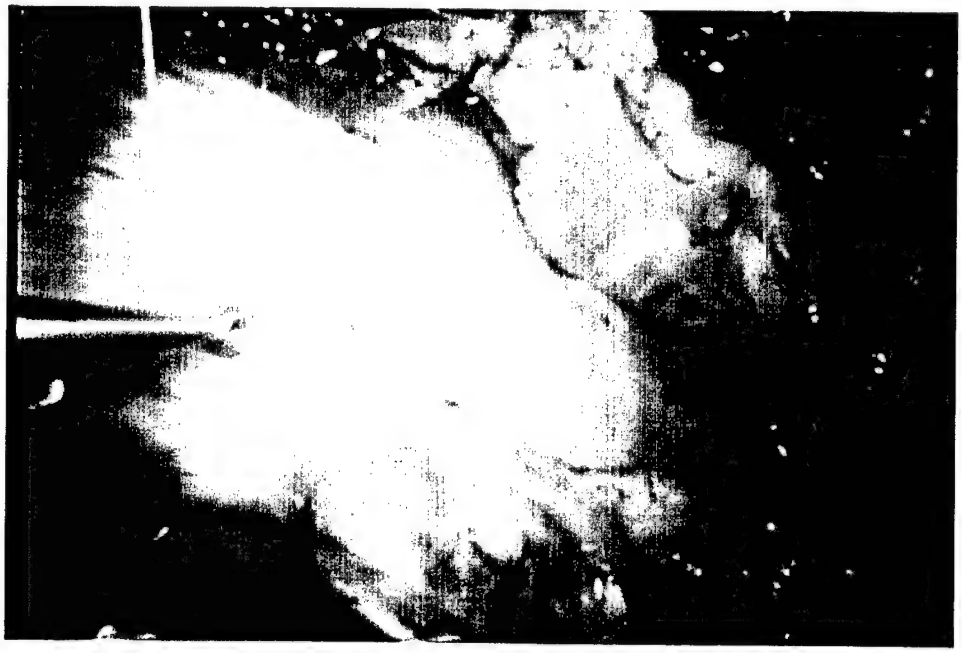


Figure 5. Suture being advanced through tissue to bring clip into proper position.

Figure 6. Clip released from suture and deployed to closed position by squeezing in forceps.

Figure 7. Successfully completed left internal mammary to coronary artery distal anastomosis with clips in place.

Task 2: Endothelial Activation Markers as Molecular Targets for Innovative, Minimally Invasive Diagnosis and Therapy in Cardiovascular Disease
Principal Investigator: Michael Gimbrone, MD, Brigham and Womens Hospital, (BWH), Boston, MA

The endothelial cells (EC) that comprise the lining of the cardiovascular system constitute a dynamically mutable interface in health and disease. In response to various inflammatory, thrombotic and atherogenic pathophysiologic stimuli (e.g., cytokines, coagulation factors, bacterial and tumor products, advanced glycation endproducts, oxidized lipoprotein components, injurious agents, biomechanical stresses), EC can undergo phenotypic modulation to a dysfunctional state that is marked by expression of "activation antigens", such as E-selectin (ELAM-1) and VCAM-1 (Athero-ELAM). The detection of soluble/shed forms of these cell surface markers in serum/plasma is already being utilized as a surrogate index of endothelial dysfunction in certain clinical studies. The team proposes to further exploit these EC phenotypic markers as molecular targets for innovative, minimally invasive diagnostic and therapeutic applications.

Key Results: Preliminary studies by the CIMIT team have demonstrated the feasibility of utilizing immunoconjugates (in the form of immunoliposomes), incorporating monoclonal antibodies to EC activation antigens, to discriminate between normal and cytokine-activated human EC in culture and to "home" to the activated EC lining of the aorta associated with early atherosclerotic lesions, following intravenous injection in experimental animals.

Specific Aim 1: To develop a reproducible, robust small rodent model of endothelial activation that combines the use of adenoviral vectors (which can efficiently mediate high-level, localized expression of a given EC activation antigen, precisely where they are introduced into the vascular system), with a simple method of introduction into an anatomically defined vascular bed.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 2: To apply radiolabeling method(s) that result in high specific activity of labeling of Fab'2 fragments of EC activation antigen-specific monoclonal antibodies, and validate the retention of specificity and avidity of binding to a cultured activated EC monolayer that expresses the target antigen(s) of interest.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report for January 1, 2001 through March 31, 2001..

Task 3: Develop a Computer Based Three-Dimensional Imaging Treatment Planning System to Drive an Endoscopically Placed, Miniature, Facial Skeletal Distraction Device.
Principal Investigator: Leonard B. Kaban, MD, DMD and Maria Troulis, MD, MGH

The significance of this project relates to the development of innovative, minimally invasive techniques for surgical treatment of patients with congenital and acquired craniomaxillofacial deformities. The combination of minimally invasive surgical techniques with the design and implementation of buried, miniature distractors guided by computer manipulated 3-D CT data, will increase operator and patient acceptance, and expand the applications of distraction osteogenesis (DO) to a variety of common craniomaxillofacial problems. There is no currently available system for surgical treatment of craniomaxillofacial deformities which links minimally invasive techniques, miniaturization, and a computer-based treatment-planning program. This concept is unique to the Massachusetts General Hospital, Skeletal Bone Research Center and the BWH Surgical Treatment Planning Laboratory project on DO.

The software developed in the first year of this project allows surgeons to visualize, explore and analyze a number of potential osteotomy alternatives in order to aid in the decision to perform minimally invasive surgery, and if so, to select the best approach (Everett et al, 2000). Specifically, when combined with existing SPL tools for anatomical segmentation and registration, this tool provides methods for: 1) Pre-surgical evaluation of 3-D diagnostic images including the visualization of 3-D data acquisitions in various modalities and the measurement of anatomical distances and angles; 2) Interactive simulation of osteotomy and planning of surgical movements of the facial skeleton: computation of reference geometry (axes, points, and planes); the simulation of an osteotomy cutting tool (geometry and movement); computation of post-surgical bone volumes; simulated movement of skeletal components with collision; computation of net movement parameters; placement of devices based on movement properties detection; 3) Post-surgical interactive evaluation of the bone movement: anatomical registration with pre-surgical images; measurement of skeletal movement against pre-surgical anatomy and against reference geometry; measurement of new bone volume.

The overall goal of this project is to apply the software application designed in the initial project for planning a variety of clinical cases. The team will use the software to determine the vector of movement, to simulate the shape and location of the osteotomy, to guide placement of the distractor and to analyze the predicted versus the actual surgical result (outcome).

Key Results: The user interface has been improved for six degree-of-freedom manipulation of 3-D graphical objects using a standard mouse. Also, an upgraded version of a 3-D axis manipulation tool was implemented in the current version of the 3-D Slicer.

Specific Aim 1: To develop a computer software application for the planning and simulation of an osteotomy and for analysis of the results. To accomplish this Specific Aim the following items will be addressed:

- To document reproducibility of the selected landmarks using both "Multiplanar" and "freehand" methods,
- To update the software and make it compatible with the new version of the "Slicer" and "Visualization Tool Kit" of the Surgical Planning Laboratory (SPL),
- To analyze skeletal changes in 25 pre and postoperative patients treated by distraction

osteogenesis,

- To apply the software prospectively for treatment planning a selected variety of distraction cases, and
- To make the program more user-friendly for clinicians and accessible as a product.

Progress: This past year, the team continued work toward conversion of the system for minimally-invasive distraction osteogenesis (MIDO) to a more user-friendly system, and one whose software is compatible with the current versions of VTK, and 3-D Slicer, the software platform upon which the planning tool is based. Recently, this work has focused on the user interface for six degree-of-freedom manipulation of 3-D graphical objects using a standard mouse. An upgraded version of a 3-D axis manipulation tool was implemented in the current version of the 3-D Slicer. Additional point selection capability was also added to the 3-D Slicer in support of this project.

Parameters of movement (POM) may be evaluated on a 3-D scatter plot. POMs include: 1) location and orientation of an axis, 2) angular displacement about the axis, 3) pitch, or translation along the axis, and 4) handedness. The POM provides a comprehensive description of the movement of a rigid structure in three-dimensional space. Trends in the relationship between radius, distance, and pitch were noted. In particular, radius is correlated with distraction distance, and pitch contributes very little to the POM. These findings are highly relevant to the question of how many distractor configurations might be needed to treat the majority of cases.

The team has been using this study to evaluate not only the range of the POM, but also the number and size of the parameter increments.

Plan: To continue to develop computer software applications for the planning and simulation of an osteotomy, and develop computer software applications for analysis of the results (outcome) of the osteotomy.

Task 4: Virtual Fixtures for Robot-Assisted Minimally Invasive Cardiac Surgery

Principal Investigator: Robert D. Howe, PhD

This project develops new computer assistance techniques to improve efficiency and increase safety in robot-assisted minimally invasive surgery. Although these techniques will be applicable to a wide variety of robotically-assisted surgical procedures, the immediate focus is coronary artery bypass graft (CABG) procedures, where robotic assistance enables minimally invasive techniques, but current robotic procedures are slow and cumbersome.

Specifically, this study develops image-guided virtual fixtures for the internal mammary harvest portion of robot-assisted CABG. In the experiments, the animal undergoes a CT scan before surgery. Small metal pins are inserted percutaneously between the ribs, to provide fixed landmarks for reference during surgery. The resulting image set is processed to define the location of the artery relative to the registration pins. In surgery, the surgeon brings the tip of a robot-mounted calibration instrument into contact with each of the pins. This permits the robot to determine the location of the pins, and thus the location of the artery from the CT image data. A virtual fixture constrains the instrument's motions, as commanded by the surgeon, to appropriate

paths adjacent to the artery. In the next implementation, a surgical macro will move the robot along the path adjacent to each artery to dissect it free of the chest wall.

Image-guided virtual fixtures and surgical macros require precise determination of the location of the IMA and registration pins. CT imaging protocol and image processing algorithms, which segment the artery from 2-D CT image data, have been developed, and its accuracy in localizing the artery has been evaluated using animal models. A virtual fixture has been implemented on the Zeus robot system, and its effectiveness has been tested using phantom models.

An abstract describing these results, "Virtual Fixtures for Robot-Assisted Minimally-Invasive Cardiac Surgery," has been accepted for presentation at the Fourth International Conference on Medical Image Computing and Computer-Assisted Intervention, Utrecht, The Netherlands, 14-17 October 2001.

Key results: CT imaging protocol and image processing algorithms were developed to extract the artery location. A virtual fixture was successfully programmed on the Zeus robot system and demonstrated its effectiveness in laboratory trials.

Change in emphasis: Tests reveal that instrument tip position accuracy is on the order of 5mm, which is inadequate for the planned procedures. The team must therefore determine the causes of the inaccuracy and rectify them.

Specific Aim 1: Develop control techniques for "virtual fixtures" and "surgical macros" that assist the surgeon in guiding robotically-positioned instruments.

Progress: Zeus programming environment, including kinematics, communications, and real-time servo routines, has been mastered. As a result, control software has successfully been implemented on the Zeus system for the first virtual fixture. Preliminary tests compared blunt dissection with and without the aid of a virtual wall. The computer-generated virtual wall confines the slave instrument tip to the region outside a specified plane. The slave instrument is free to follow the master instrument in all three dimensions outside the proscribed region, while it follows only lateral motions on the wall surface if the master controller is moved within the region. Results with four subjects showed that the virtual wall reduced task completion time by over 27% and eliminated excursions beyond the desired region.

Plan: This task is largely finished; however, tests have revealed that robot position accuracy is approximately +/- 5 mm. Compliance in the instrument holder and distal joints seems the most likely cause, and redesign of the these components will be attempted.

Specific Aim 2: Develop image-guided fixtures and macros that use preoperative 3-D patient images to help direct instruments to the appropriate tissues.

Progress: CT imaging protocol and image processing algorithms have been developed to extract the registration pin and internal mammary artery locations. The developed algorithms segment the centerlines of arteries and centroids of registration pins from 2-D CT image data. The accuracy of registration was on the order of 2 mm in animal models.

Plan: Output from the developed image processing will be cross-checked by other segmentation algorithms, developed by other research groups of one of the Co-PIs (Grimson). This software,

SLICER, segments artery and registrations pins from 3-D image data constructed from 2-D CT images. This will allow the localization of registration pins and internal mammary artery based on anatomical landmarks. More precise localization may also be achieved by enhancing robot position accuracy noted above.

Specific Aim 3: Measure the performance of the enhanced system in surgical procedures on animal models in terms of: (i) the improvement in control of surgical tasks; and (ii) the precision attainable with these techniques.

Progress: No activity this year.

Plan: This activity follows accomplishment of the previous two Specific Aims.

2.3 IMAGE GUIDED THERAPY

Virtually every device that supports minimally invasive procedures relies on processed images. These images provide preoperative data and guide surgery. While already useful for brain, spine and musculoskeletal surgery, current systems have limitations: the integration is awkward, they slow down the procedures they are intended to facilitate, and data preparation in clinical setting sis too time consuming. To overcome such limitations Image Guided Therapy Enabling Technology is developing robust and flexible algorithms that incorporate knowledge about anatomy and pathology and provide intuitive user interfaces.

Task 1: MRI-guided Focused Ultrasound Treatment of Breast Cancer

Principal Investigator: Ferenc Jolesz, MD, BWH

The overall goal of this project is to develop a magnetic resonance imaging (MRI) guided focused ultrasound system for thermal coagulation of breast cancer. The first accomplishment for making clinical breast treatments practical is to develop and test phased array ultrasound applicators that allow the focal spot size to be increased. This is needed for two reasons: A large focal spot allows the tumors to be coagulated in a shorter time, making the treatment time practical. It also reduces the nonuniformities in the temperature field thus assuring better treatment response. The team has developed and tested a phased array applicator that is now implemented in a clinical MRI guided focused ultrasound system for breast cancer treatments. The team has also been able to test the ability of MRI-derived thermal dosimetry to determine tissue coagulation *in vivo*. These methods have now been implemented in the control of clinical MRI guided focused ultrasound in several institutions and are also being tested for use during MRI monitoring of thermal coagulation of tumors using laser fibers. Overall, the team has made significant progress that will make noninvasive MRI guided thermal coagulation of breast tumors practical for clinical testing.

To study this hypothesis in a clinical setting the team needs to develop our sonication and MRI thermometry methods for practical treatments and to test them in animal experiments.

Key Results: During this past year, the phased array technology was translated to pre-clinical testing in an animal model. The team tested a 208 channel completely integrated system by sonicating rabbit tissues and implanted tumors. This system was completed based on the tests and is now ready for clinical trials.

Specific Aim 1: Develop treatment-plan procedures utilizing 3D-MRI information to determine the target volume and execute treatment:

- To evaluate the feasibility of inducing temperatures between 60 and 100°C in tissue volumes required for breast cancer treatments during a 10-60 second sonication.

Progress: The several phased array systems were tested and results used to develop the final 208-channel phased array system in collaboration with a commercial manufacturer. This system was tested in rabbits. The results are good, demonstrating the ability of the system to coagulate tumor tissue similar in size and location to breast cancer.

Plan: To move this technology forward to clinical trials.

Specific Aim 2: Study the accuracy of MRI-derived temperature history for calculating the thermal exposure of tissue.

The goal of this Specific Aim is to test and evaluate the feasibility of using MRI thermometry to estimate the temperature and thermal dose induced by the sonications and to test its accuracy. The team used *in vivo* animal tissues for these tests.

Progress: During this past year, the team has used the *in vivo* sonications of rabbit thigh muscle to test the thermometry. The MRI post treatment imaging (and histology) demonstrated that a narrow threshold value can be found based on the MRI derived thermal dose that can be used as indicator of successful treatment. This will be very important for the online control of tumor treatments.

Plan: To continue to develop and refine MRI-derived temperature technology.

Specific Aim 3: Establish the thermal exposure required to assure complete tumor coagulation.

Progress: Multiple sonications with phased arrays were performed *in vivo* in rabbit tissues while mapping the temperature using MRI. The MRI contrast enhanced scans were used to evaluate the coagulated volume. These MRI contrast enhancement and histology demonstrated that complete tissue volume coagulation can be induced by these sonications while evaluating the thermal dose using online MRI thermometry.

Plan: To continue to develop and refine MRI-derived temperature technology for *in vivo* tumor coagulation.

Specific Aim 4: To test Specific Aim 3 in implanted rabbit tumors.

Progress: Experiments using MRI contrast agent to help to target rabbit tumors were continued. Its impact on the thermal imaging and on the MR images obtained after the sonications are currently being investigated. After the sonications and the MR imaging, the animals were allowed to survive to evaluate tumor regrowth by using MR imaging. These experiments demonstrated that when adequate thermal exposure covered the whole tumor then there was no regrowth of the tumor.

Plan: To continue to develop and refine MRI-derived temperature technology in implanted rabbit tumors.

Specific Aim 5: To evaluate the influence of fat and tissue motion on the MRI dosimetry.

Progress: Fat suppression of the proton resonant frequency shift based thermometry pulse sequences was found to be useful in tissues that had mixed fat in the tissue. Without the fat suppression the MRI measured temperature was not correct. In pure fat the team has demonstrated that T1-weighted Fast Spin Echo sequence shows the location of the hot spot and indication of the temperature elevation. In addition motion insensitivity was demonstrated with internally referenced pulse sequences.

Plan: The plan is to continue with the tumor sonications and further test the fat suppression and motion correction methods.

Task 2: Early Detection and Ablation of Epithelial Cancers
Principal Investigator: Norman S. Nishioka, MD, MGH

This study will investigate the use of aminolevulinic acid (ALA) to enhance the fluorescence signal from dysplastic cells in the esophagus in the specific case of Barrett's esophagus. High-grade dysplasia in Barrett's esophagus has been known to progress into adenocarcinoma. Patients diagnosed with Barrett's usually require long-term endoscopic surveillance. While Barrett's epithelium is easily detected on endoscopy, regions of dysplasia cannot be easily visualized with white light. The results of previous studies have demonstrated the potential of this method to identify and localize pre-malignant regions of the esophagus that cannot be seen during routine endoscopy. This study will determine the accuracy of orally-administered ALA in marking dysplasia and develop, test and improve the endoscopic system for fluorescence detection in the esophagus.

Subtask 1: ALA Enhanced Fluorescence Imaging of Barrett's Epithelium

Specific Aim 1: Determine the accuracy of orally administered ALA for marking dysplasia occurring in Barrett's esophagus.

Progress: Project completed. See Quarterly Progress Report for the period January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Subtask 2: OCT Imaging of Esophageal Lesions
Principal Investigator: Norman S. Nishioka, MD, MGH

The goal of this project was to determine the clinical utility of OCT for imaging lesions in the gastrointestinal (GI) tract. Modern gastrointestinal endoscopy is a potent diagnostic and therapeutic technique for the management of a wide variety of GI disorders. However, one limitation of conventional endoscopy is the inability to visualize structures beneath the mucosal surface of the GI tract. The advent of endoscopic ultrasound has made it possible to visualize subsurface structures in many settings, but the instrumentation is expensive and the spatial resolution is limited by the transducer operating frequency (<30 MHz). Optical coherence tomography (OCT) is an alternate technique for obtaining high resolution cross-sectional images of tissue. The operating principles of OCT are analogous to ultrasound except that light waves are used to image tissue rather than acoustic waves. The spatial resolution of OCT is approximately 10 times better than that of the best ultrasound devices.

Specific Aim 1: Perform a pilot trial of OCT in unselected patients undergoing upper endoscopy to assess the spatial resolution and clinical usability of the present system.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Task 3: Segmentation of Bone from CT and Vessels from MRA Data
Principal Investigator: Carl-Fredrik Westin, PhD and Ron Kikinis, MD, BWH

The broad goal of this Enabling Technology project is to improve the way that information from medical image data is extracted. The project is a continuation of our ongoing effort to develop new technologies to improve efficiency and specificity in creating patient specific anatomical 3D models for surgery simulation, surgical planning, and image guided intervention.

Key Results: The team continued to incorporate recent results in segmentation of medical data using geodesic active contours into the existing segmentation scheme from last year based on adaptive filtering. Active contours in 3D mean that a 2D surface is evolved iteratively in 3D. This work builds on the experience in the Surgical Planning Laboratory at Brigham and Women's Hospital, over the past decade, in segmenting clinical data for neurosurgical applications. Further, the team has been developing a user-steered segmentation algorithm based on the livewire paradigm. Livewire is an image feature driven method that finds an optimal path between user-selected image locations, thus reducing the need to manually define the complete boundary. The team has introduced a new image feature, based on local phase, which describes local edge symmetry independent of absolute gray value. The phase is a natural bi-product from the filters used in the adaptive filtering scheme presented last year in the project. Because phase is amplitude invariant, the measurements are robust with respect to smooth variations, such as bias field inhomogeneities present in all MR images. In order to enable validation of the phase-wire segmentation software, a system has been created that continuously records user interaction and automatically generates a database containing the number of user interactions, such as mouse events, and time stamps from various editing modules. The team has conducted validation trials and obtained expert opinions regarding its functionality.

Specific Aim 1: To implement a data enhancement scheme for segmentation of bone from CT and vessels from MRA.

Progress: The adaptive filtering algorithm is fully implemented in optimized C-code. The code was parallelized by using routines for shared-memory threads on SMP hardware. The code also performs well on a single CPU machine. The computational time is less than 15 minutes for a typically sized 3D data set (256x256x60 voxels) with a memory model of less than 100 Mb. Figure 2 (right) shows an example of bone segmentation from CT that uses this technique.

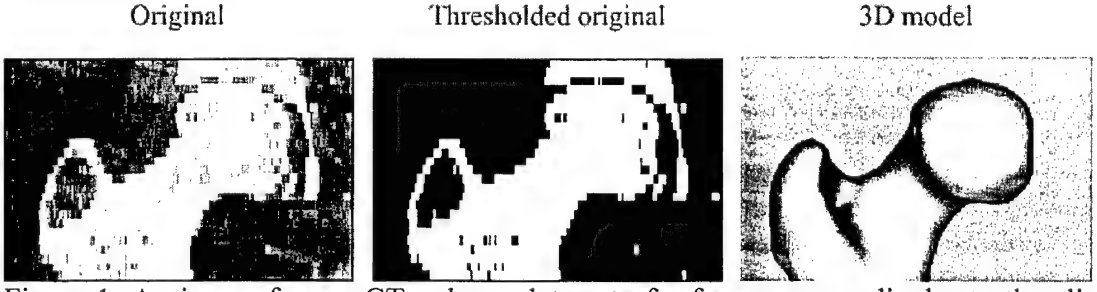


Figure 1. An image from a CT volume data set of a femur, perpendicular to the slice direction (left) and the result of thresholding the original data set (middle). Notice that the joint space is not separated due to the large size of the voxels in the slice direction. By filtering the CT data set with affine adaptive filters developed by the team during Year 1 of this project, the data set is

resampled to higher sampling density (smaller voxels), and the joint space can easily separated and a 3D femur model can automatically be generated (right).

Plan: Project completed.

Specific Aim 2: Optimization and Validation: to quantitatively validate and optimize the automated segmentation method results:

- Optimization and Validation: validation of phase-wire segmentation software.

Progress: The team found that the parameters that most affected the overall performance of the adaptive filtering scheme are: 1) the transition frequency between the low-pass and the high-pass filters, and 2) the noise level parameter that defines noise and image structure in the data. Selection of the data for validation was performed in close collaboration with our clinical partners. A few slices in each of the data sets were selected and then, critical areas on these slices were outlined. This provided a gold standard that was used as a criteria for finding optimized parameter settings. The semi-automatic procedures used in our Surgical Planning Laboratory today (thresholding, connectivity, cleaning and mending in volume editor) served as a reference. In the validation study, the team also compared the segmentation of different resolution scans. MRA scans were acquired with different resolution, both in a 0.5T MR scanner (GE Open Magnet at BWH) and a 1.5T MR scanner (GE system at BWH). All scans were carefully, manually segmented by a neurosurgeon.

The preliminary results using local phase as the main driving force are promising and support the fact that phase is a fairly stable feature in scale space. The method has been found intuitive to use. In order to enable validation of the phase-wire segmentation software, a system has been created that continuously records user interaction and automatically generates a database containing the number of user interactions, such as mouse events, and time stamps from various editing modules. Analysis of the logged information has shown that great variability exists in the segmentation times, partially due to users' segmentation style, learning curve, and type of image data. Consequently, a complete comparison of the phasewire and manual system is difficult. Perhaps the most reliable indication of the systems performance can be found in the opinions of doctors who have used it. Doctors remarked that the three-dimensional models created using the phasewire system are more anatomically accurate than the ones produced with the manual method, where both segmentations were performed in approximately the same time. This is likely due to the smoothness of the contour obtained by the phase wire, in comparison with the contour obtained with the manual method.

Plan: The team plans to further validate the phasewire database containing the recorded users interactions with the system.

Specific Aim 3: Extend our current implementation of adaptive filtering to incorporate interpolation to a finer grid.

Progress: The team has compared the effect of locally shifted adaptive filters for resampling, with sinc interpolation using zero-padding in the Fourier domain with subsequent adaptive filtering. The advantage of the zero-padding technique is that the implementation can be made more efficient. During this second year of the project, the adaptive filtering code developed during the first year of this project has been extended with a zero-padding module, to handle the

resampling of image volume data to higher resolution. The implementation is based on zero-padding of the data in the Fourier domain. Since the zero-padded data is filtered with directional filters before being combined and transformed back to the spatial domain, many of the ringing artifacts normally associated with sinc interpolation are greatly reduced. Further, as a part of the test-bed and validation effort, computer generated vessel phantoms were created containing different levels of noise. An important finding was that filtering of these data sets, with artificially inserted stenoses, the local diameter of the vessels was not markedly altered and the degree of stenoses was not changed. The team has also investigated how different data border extension techniques affects the ringing artifacts introduced by the sinc interpolation. These artifacts were considerably reduced when a border extension using a Gaussian envelop with data mirroring with cyclic averaging was introduced. Recently the team has applied the adaptive filters to MRI knee data with very encouraging results for defining boundaries of the cartilage (Figure 2).

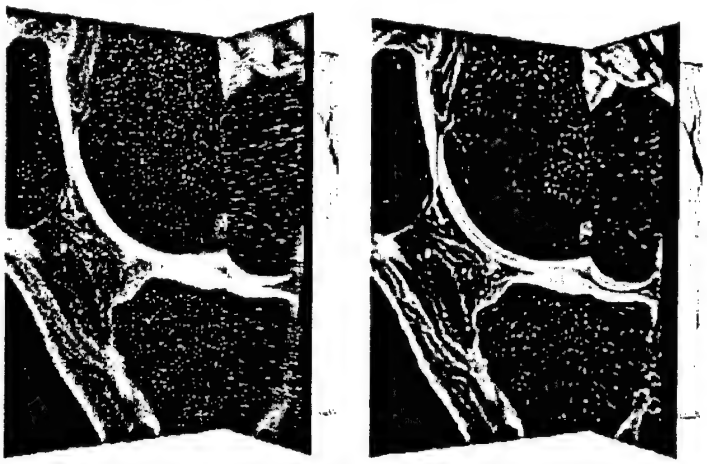


Figure 2. Fat saturated SPGR MRI data set of the knee with a voxel size of 0.2x0.2x1.5 mm (left) and the result after adaptive filtering to 0.2x0.2x0.21mm (right). Notice the improved visibility of the cartilage in the filtered image.

Plan: To implement a new class of optimized filters for the adaptive filtering procedure. The team plans to investigate a new class of filters for the local structure estimation. By using a Wiener filter in both the estimation and the synthesis step, it is predicted that fewer user control parameters will be necessary, which in turn will facilitate the use of the system. Further, the team plans to investigate the impact of optimization of the filter functions in the spatial domain and in the frequency domain simultaneously. It is predicted that the new filters will perform well in a wider range of parameters, which also will make user interaction easier.

Specific Aim 4: To develop a segmentation model based on the team's experience on adaptive filtering and surface evolution.

Progress: The team has recently developed the CURVES system, which segments vessels from MRA images by evolving an initial estimate toward the true structures in the image using the codimension-two regularization force. This year the team has been focused on tuning a pipeline consisting of adaptive filtering of the vessel data followed segmentation by the CURVES system. In addition to segmenting vessel data some effort was put on segmentation of bone from CT using the surface evolution technique CURVES is based on. One problem with the current implementation is that the driving image force is purely based on image gradients. This means

that all structures with high contrast attract the surface and a structure like skin is equally attractive as bone for the evolving surface. To solve this problem, further enhancements during this year included the incorporation of a spatially limiting mask based on the gray-level value of the data (Figure 3).

Initial surface | Codimension-one regularization | Spatially constrained adaptive codimension

Figure 3. Bone segmentation: Initial boundary by thresholding a CT volume (left), unconstrained surface evolution will find the skin (middle), and spatially constrained evolution will find the optimum bone boundary (right).

Several avenues for incorporating information of local image structure to the surface evolution equation have been investigated. In addition to the local structure tensor developed for that adaptive filtering scheme, local structure from gradient outer products has been implemented and compared (Figure 4).

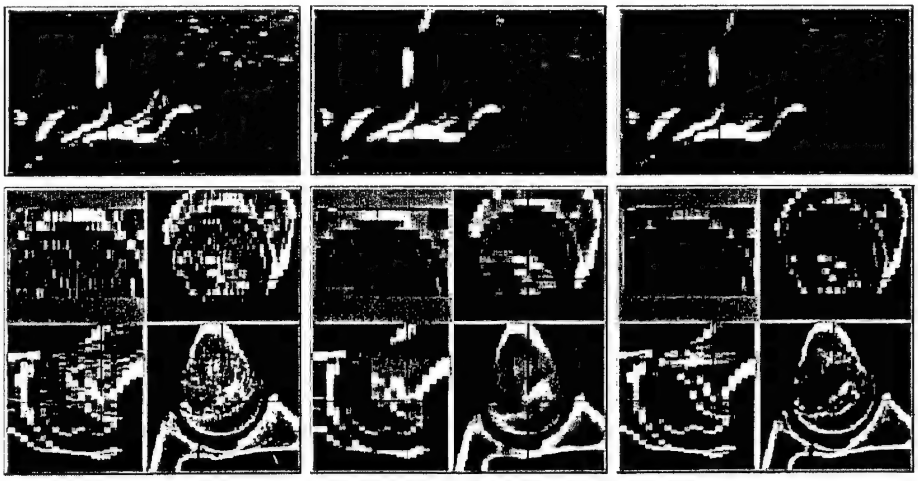


Figure 4. Top row shows data from phase contrast MRA and bottom row shows CT data of a femoral joint. The original data (left) was restored using both anisotropic diffusion (middle) and anisotropic adaptive filtering (right).

Further, the team has developed a user-steered segmentation algorithm based local phase, an image feature derived form the filters used in the adaptive filtering scheme introduced last year in this project. The new method is based on the livewire paradigm. Livewire is an image feature driven method that finds an optimal path between user-selected image locations, thus reducing the need to manually define the complete boundary. Local phase is an image feature that describes local edge symmetry independent of absolute gray value. The phase-based livewire segmentation method has been integrated into the 3D Slicer, the Surgical Planning Lab's

platform for image visualization, image segmentation, and surgical guidance. The 3D Slicer is the laboratory's program of choice for manual segmentation. Any time during an average day, one would expect to find ten people doing segmentations using the Slicer, which shows that any improvement on manual methods will be of great utility. These results have also recently been presented at the 2001 MICCAI conference, the leading conference in medial image analysis.

Plan: To validate the new local structure estimators and to revisit the basic implementation of the evolution algorithm. The current computational time for the CURVES system is approximately 40 min. The time needs to be reduced by approximately a factor of ten to useful in a clinical setting (and be comparable to the adaptive filtering step which last year was reduced to 4 min.).

Specific Aim 5: To develop a symbolic description of vessel anatomy based on vessel mid-lines, vessel diameters and branching points.

Progress: The team has started to develop a description of vessel anatomy based on vessel mid-lines, vessel diameters, and branching points. This provides a powerful description of anatomy useful for interaction with higher level systems, such as a surgical simulator. Once the data has been segmented, the distance function from the codimension-two surface evolution provides additional information that can be exploited. The team has identified the ridges of the negative distance values (inside the vessels). This is a new, robust way to find the skeleton of the vessel tree (Figure 5). Skeletons are useful for things such as anatomical labeling, automatic path generation for virtual endoscopic fly-through simulations, and estimation of vessel length needed for placements of stents.

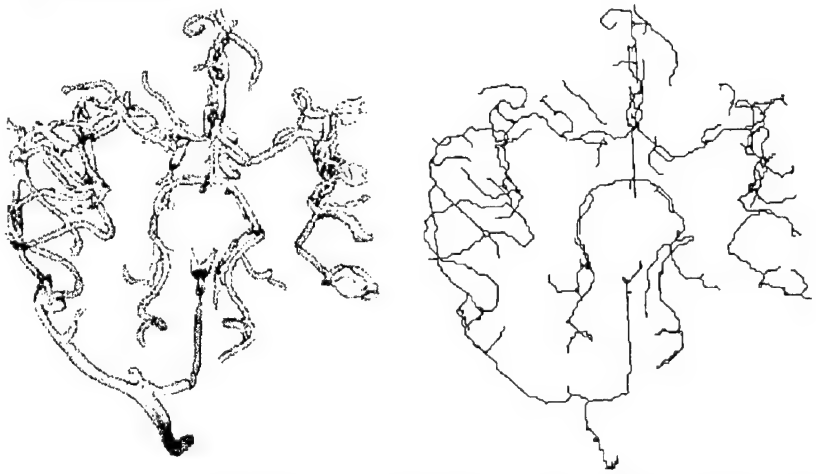


Figure 5. Segmentation of vasculature from phase contrast MRI data (left) and reconstructed center-lines (right).

Plan: To validate the new center-line extraction method by comparing to alternative methods in the literature.

Task 4: Real-time Registration of Intra-operative Ultrasound with Pre-operative CT/MR for Image Guided Therapy

Principal Investigator: Eric Grimson, PhD, Massachusetts Institute of Technology (MIT), Cambridge, MA

The utility of minimally invasive therapy depends, in no small measure, on the ability to precisely deliver therapy to the targeted site. The efficacy of image guided therapies is now well documented in the literature for such applications as tissue biopsy, cryotherapy, brachytherapy, and energy delivery. For the most part, however, image guidance requires expensive intra-operative equipment (e.g., intra-operative MRI), ionizing radiation (e.g., fluoroscopy, CT), or is limited to surface (e.g., luminal) imaging of areas accessible through videoendoscopic tools. Although inexpensive, non-ionizing, subsurface-capable, and portable, ultrasound imaging has not found the widespread usage that one might expect, due largely to the poor-contrast, specular noise, and unintuitive nature of ultrasound imagery. In this proposal the team aimed to demonstrate a novel new method for improving the visualization quality of intra-operative ultrasound imagery. Specifically, because of the overwhelming preference of users for high-contrast CT/MR imagery, and since such imagery are frequently acquired pre-operatively, the team aimed to demonstrate the ability to register these high contrast pre-operative imagery to yield the same view as the intra-operative ultrasound. The approach enabled, effectively, an intra-operative CT/MR imagery from which image guidance can be performed, but without incurring the costs and risks associated with continuous CT/MR imaging.

The goal of this project was to demonstrate the ability to deform pre-operative CT/MR images, in a non-real time manner, so that point-to-point correspondence to an intra-operative ultrasound can be obtained. This was readily achieved in the first phase of the project for analog ultrasound and CT images of abdominal region containing liver tumor. The demonstration involved enhancement of the ultrasound and CT images, extraction of robust edge-feature images, registration of the edge-feature images involving estimation of the mapping function between the two different types of images and the fusion of the two images. Similar demonstration was attempted on the digital ultrasound data in the second phase of the program. Application of the edge feature extraction procedure, developed for the analog ultrasound data, yielded however disappointing results. Digital ultrasound data did not have enough contrast in comparison to the analog data to yield robust edges of sufficient strength for use in image registration. The efforts were then directed towards the modification of the image enhancement and edge-feature extraction algorithm that would be geared towards the extraction of weak edges. Modification of the algorithm based on prescribed anisotropic smoothness characteristics for the enhanced image and edge-feature images however did not produce any improvement in the edge extraction. It was felt that any further attempts for enhanced feature extraction for a data with inherently weak contrast would lead to more involved and complex processing and thus detract from the ultimate goal of real time implementation.

Key Results: The 3-D CT/MR registration of prostate data was achieved by using point correspondences and a polynomial warping algorithm to create a dense deformation field, which was used to transform the patients' MR data to an atlas CT image. The team created an error analysis algorithm in which the team placed a set of corresponding points on reserve and used a separate set of points to compute the registration. The interpolated dense deformation field was then applied to the reserve points and error analysis was performed. The parameters which the team varied were polynomial order, quantity of point set data, and type of point distribution. Based upon our calculations, the team concluded that the 2^{nd} order polynomial was sufficient for

minimizing the error in the registration calculations. This implies that fewer corresponding points can be used to compute an accurate registration. Also, the team showed that a horizontally inclined correspondence distribution produced the minimum mean calculation compared to using any of the distributions tested in our research.

Specific Aim 1: Demonstrate the ability to register pre-operative CT/MR, in a non-real-time manner, so that point-to-point correspondence to an intra-operative ultrasound can be obtained.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 1.1: Select a set of surface points from MR/CT, and use ICP to match the points to edges in ultrasound. This result provides an initial correspondence between the two data sets.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 1.2: Explore numerous variations on polynomial warping methods which included the following: variation of the number of corresponding points; variation of polynomial order; variation of the point set distribution; and error verification to measure sensitivity in our computed registration errors.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

2.4 TISSUE ENGINEERING

The field of Tissue Engineering is now maturing and undergoing explosive growth. Virtually every tissue and organ of the body has been studied. Many tissue engineering technologies are becoming available for human use. Over time, several techniques to engineer new living tissue have been studied. Technologies include the use of growth factors to stimulate wound repair and regeneration, techniques of guided tissue regeneration using non-living matrices to guide new tissue development, cell transplantation, and cell transplantation on matrices. Recent studies in stem cell biology has led to studies of populations primordial cells, stem cells or embryonic stem cells to use in tissue engineering approaches.

Task 1: Degradable Conductive Polymers
Principal Investigator: Robert Langer, ScD, MIT

Electroactive polymers, which constitute a unique class of synthetic polymers, possess the ability to inter-convert chemical, mechanical, thermal and optical perturbations into tiny electrical currents. This property can be exploited to play an important role in the interfacing of the external environment with biological systems. Electronically conductive polymers are especially attractive in that, they can not only be employed as guidance channels or substrates for tissue culture but can also potentially be utilized as a medium to subject the adhered tissue (cells) to an electrical stimulus. The team has shown that electrical stimulation of neuronal and mesenchymal progenitor cells adhered to conductive oxidized polypyrrole (Ppy) substrates, in presence of soluble morphogens and growth factors can aid in the lineage specific differentiation of these cells. In its traditional chemical form oxidized Ppy is non-degradable and is minimally processible. A more processible and bioerodible Ppy would be particularly important for applications wherein a PPy coating is used to alter the surface characteristics or tissue response to a prosthetic for a well-defined period such as coating of a vascular stent to minimize smooth muscle proliferation and restenosis. One can also envision coating of metal or carbon composite or other polymeric orthopedic prosthesis with conductive polymers such as polypyrrole to improve tissue compatibility and adherence of the implant to surrounding tissue. Furthermore, from a tissue-engineering standpoint, it would be ideal if the conductive polymer matrix served as a template for the desired period and underwent degradation thereafter thus eliminating any potential long-term undesirable tissue response.

Key Results: This year the team proposed a novel approach to the creation of bioerodible polypyrrole (Ppy). In this novel paradigm the rate of erosion of Ppy is controlled by the hydrolysis and ionization of pendant groups followed by the solubilization of Ppy oligomers. The team has verified the hypothesis that solubilization of Ppy solid and thin film substrates, via ionizable side chain moieties, can occur under physiological conditions.

Specific Aim 1: To synthesize degradable analogs of the conductive polymer PPy, as well as water soluble analogs, and to study the degradation and cytocompatibility characteristics of these polymers.

Progress: During the past year the team explored a unique approach to the synthesis of polypyrrole (Ppy). It is based on the premise that the introduction of an ionizable alkyl moiety in the beta-position of the pyrrole repeat unit will result in the solubilization of the polymer under certain conditions. The team has reported in our last progress report that both electrochemically

and chemically synthesized Ppy, containing pyrrole units bearing butyric acid moieties (4-(β-pyrrolyl)-butyric acid (Ppy-acid)) in the beta position were capable of undergo erosion and dissolution under basic conditions. The team further showed the erosion rate was diminished upon decrease in pH suggesting that the ionization of the carboxylic end group was responsible for the solubilization of Ppy units. This is consistent with what was proposed.

During the past three months the team has been studying the mass loss of these novel materials and comparing the erosion behavior of the free acid to the methyl ester derivative (1-methyl-4-(□-pyrrolyl)-butyrate (Ppy-ester)). If the erosion/dissolution of the Ppy is due to the carboxylic acid side chain, then the esterification of the acid group should result in a marked diminution in erosion behavior. To test this hypothesis PPy-acid and Ppy-ester pressed pellets were placed into 10 mL of 0.1 M HEPES buffer (pH 7.2) and incubated at 37 °C on an orbital shaker and the mass loss in these pellets was followed as a function of time. For experiments employing Ppy-acid, the buffer media was removed for absorbance measurements and replaced with fresh media every 2-5 days while for the PPy-ester pellets the buffer media was collected and analyzed only every 7-14 days. The mass loss of the pellets was analyzed at selected time points by removing the incubation media, washing the pellets with de-ionized water (3 × 5 mL), and subsequently freeze-drying each pellet to constant mass. All experiments were performed in triplicate.

It was observed that pellets formed from hydrophobic methyl ester-functionalized Ppy (i.e., Ppy-ester) eroded much more slowly than their acid-functionalized counterparts (50 mg, diameter = 8 mm, thickness = 1 mm) at pH 7.2 (Figure 1) as hypothesized. In fact the mass loss from pellets of Ppy-acid was 27% after 80 days of incubation at 37 °C, while the mass loss from pellets of Ppy-ester was only 6%. These results parallel an increase in pyrrole oligomer concentration in solution over time (as determined by UV/vis spectroscopy, Figure 1). The disparity in the erosion/dissolution rates between the acid and ester derivative suggests a means of tailoring erosion rates through judicious co-polymerization of the two pyrrole monomers.

Plan: In the coming quarter the team plans to study the cytocompatibility of these novel Ppy films in order to evaluate their usefulness in Tissue Engineering applications.

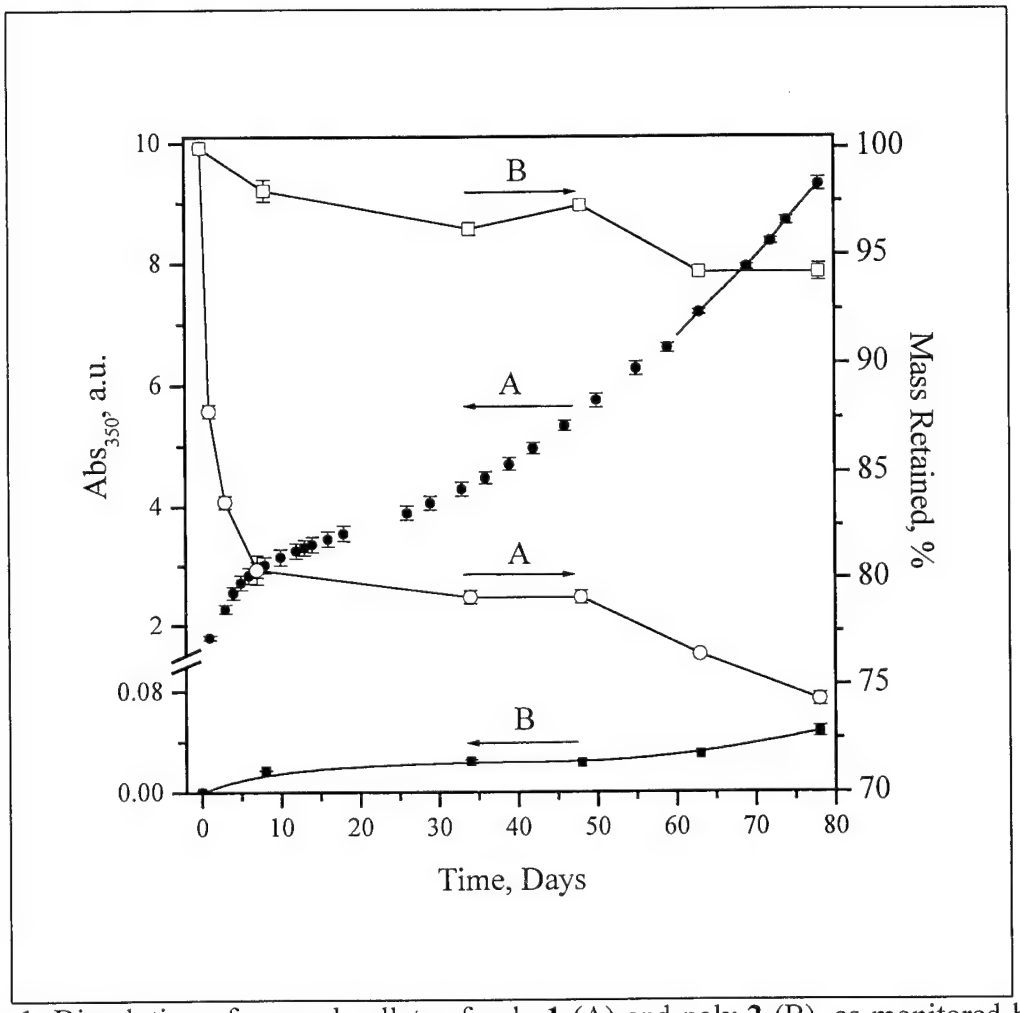


Figure 1. Dissolution of pressed pellets of poly-**1** (A) and poly-**2** (B), as monitored by solution UV/vis absorbance and mass loss.

Task 2: Polymer-based Gene Delivery Platform
Principal Investigator: Robert Langer, ScD, MIT

Safe and effective pharmaceutical delivery systems for DNA will need to be developed in order for the field of gene therapy to advance further into the clinic. For local therapeutic levels of protein to be generated, high levels of gene expression within a desired subset of cells is generally required. To this end, the local delivery of gene therapeutics via minimally invasive modalities, such as catheters or endoscopes could lead to important advances, because these techniques can be used to administer DNA (and thus therapeutic protein) at desired sites rather than administering them systemically.

The long-term goal of this project has been to create a safe synthetic polymeric gene delivery system with high transfection efficiency for local delivery of plasmid DNA. The work conducted toward Specific Aim 1 of the current grant period has been directed toward the continued development of new polymeric "proton sponge" materials and the development of a mechanistic understanding through which these materials mediate transfection. Progress on this

Aim has been limited by the departure of two members of the team, but the team has initiated a collaborative effort with investigators at the University of California at San Francisco to investigate their most promising polymers for the delivery of therapeutic HIV vaccines in mouse models. The work conducted toward Specific Aim 2 has been directed toward the development of new pH-responsive materials for enhanced intracellular delivery, and has been the primary focus of work conducted during this Year.

Key Results: The team reports the first accelerated discovery approach for finding synthetic transfection vectors. This built on the team's previous work in synthesizing poly(β-amino ester)s. Importantly, initial results suggest several new polymers in the libraries generated have higher transfection efficiency than existing synthetic vectors in cell based assays.

Specific Aim 1: To synthesize a polymer-based gene delivery system that on the molecular level mimics viruses. To accomplish this Specific Aim the following item will also be addressed:

Progress: As previously stated, progress toward this Specific Aim has been limited by the departure of two of the team members. The team initiated a collaborative project with Prof. Chris Locher in the Department of Virology at the University of California at San Francisco. The two teams are conducting a head-to-head *in vivo* comparison of naked DNA, liposome formulations, and the most promising polylysine-graft-imidazole polymers employing a therapeutic HIV vaccine and a mouse model.

Plan: To continue the collaboration with the Department of Virology at the University of California at San Francisco.

Specific Aim 2: To synthesize new degradable polymers for use as gene transfer vectors, and to investigate the degradability, cytotoxicity, and ability of these polymers to condense and/or encapsulate DNA into particles suitable for transfection.

Progress: Poly(β-amino ester)s are hydrolytically degradable, condense plasmid DNA at physiological pH, and are readily synthesized via the conjugate addition of primary or secondary amines to diacrylates. An initial screen of model polymers identified these materials as potential gene carriers and demonstrated that structural variations could have a significant impact on DNA binding and transfection efficacies. The team reasoned that this approach provided an attractive framework for the elaboration of large libraries of structurally-unique polymers for several reasons: 1) diamine and diacrylate monomers are inexpensive, commercially available starting materials, 2) polymerization can be accomplished directly in a single synthetic step, and 3) purification steps are generally unnecessary as no byproducts are generated during polymerization.

The paucity of commercially available bis(secondary amines) limits the degree of structural diversity that can be achieved using the above synthetic approach. However, the pool of useful, commercially available monomers is significantly expanded when primary amines are considered as potential library building blocks. Because the conjugate addition of amines to acrylate groups is generally tolerant of functionalities such as alcohols, ethers, and tertiary amines, the team believed that the incorporation of functionalized primary amine monomers into their synthetic strategy would serve to broaden structural diversity. Diacrylate monomers A-G

and amine monomers 1-20 were selected for the synthesis of an initial screening library (Figure 1).

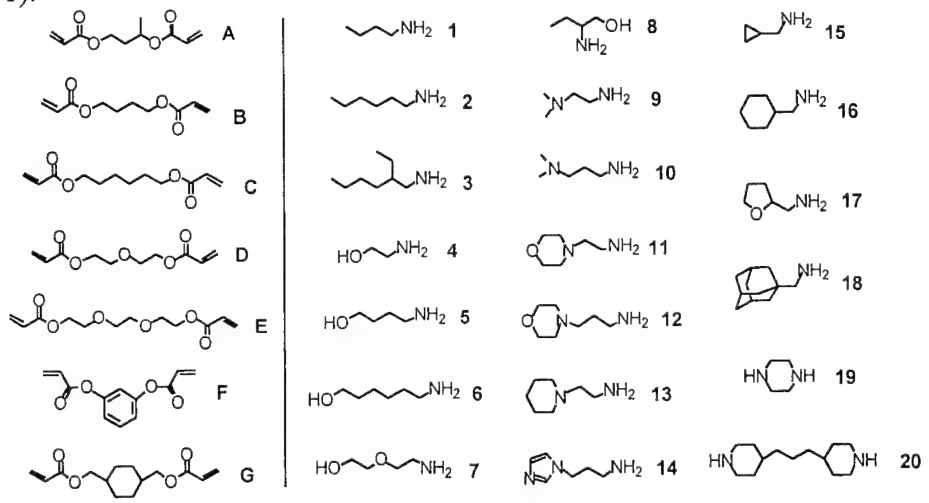


Figure 1: Diacrylate (A-G) and amine (1-20) monomers chosen for the synthesis of an initial screening library.

The size of the library constructed from this set of monomers (7 diacrylates x 20 amines = 140 structurally-unique polymers) was chosen to be large enough to incorporate sufficient diversity, yet small enough to be practical without the need for automation in the team's initial studies. It was unclear at the outset whether a polymer such as G16 (formed from hydrophobic and sterically bulky monomers G and 16) would be water-soluble at physiological pH or be able to condense DNA sufficiently. However, monomers of this type were deliberately incorporated to fully explore diversity space, and in anticipation that this library would ultimately be useful as a screening population for the discovery of materials for applications other than gene delivery.

Polymerization reactions were conducted simultaneously as an array of individually labeled vials. Reactions were performed in methylene chloride at 45°C for 5 days, and polymers were isolated by removal of solvent to yield 600-800 mg of each material. Reactions performed on this scale provided amounts of each material sufficient for routine analysis by GPC and all subsequent DNA-binding, toxicity, and transfection assays. A survey of 55% of the library by GPC indicated molecular weights ranging from 2000 to 50 000 (relative to polystyrene standards). As high molecular weights are not required for DNA-complexation and transfection (as shown below). this library provided a collection of polymers and oligomers suitable for subsequent screening assays.

Of the 140 members of the screening library, 70 samples were sufficiently water-soluble (2 mg/mL, 25 mM acetate buffer, pH = 5.0) to be included in an electrophoretic DNA-binding assay (Figure 2). To perform this assay as rapidly and efficiently as possible, samples were mixed with plasmid DNA at ratios of 1:5 and 1:20 (DNA/polymer, w/w) in 96-well plates and loaded into an agarose gel slab capable of assaying up to 500 samples using a multi-channel pipette. All 70 water-soluble polymer samples were assayed simultaneously at two different DNA/polymer ratios in less than 30 minutes. As shown in Figure 2, 56 of the 70 water-soluble polymer samples interacted sufficiently with DNA to retard migration through the gel matrix (e.g., A4 or A5), employing the 1:20 DNA/polymer ratio as an exclusionary criterion. Fourteen polymers were discarded from further consideration (e.g., A7 and A8), as these polymers did not complex DNA sufficiently.

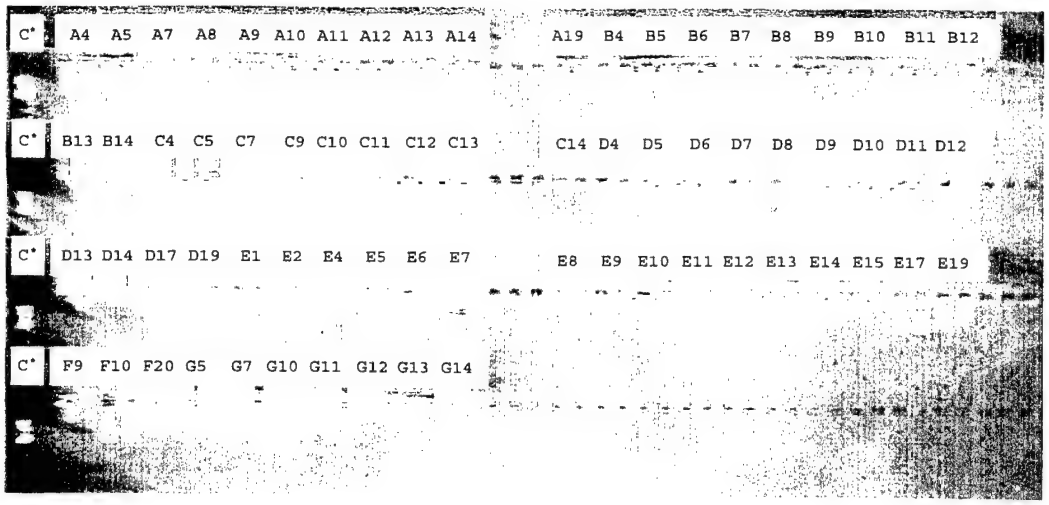


Figure 2: Gel electrophoresis assay used to identify DNA-complexing polymers. Lane annotations correspond to the 70 water-soluble members of the screening library. For each polymer, assays were performed at DNA/polymer ratios of 1:5 (left well) and 1:20 (right well). Lanes marked C* contain DNA alone (no polymer) and were used as a control.

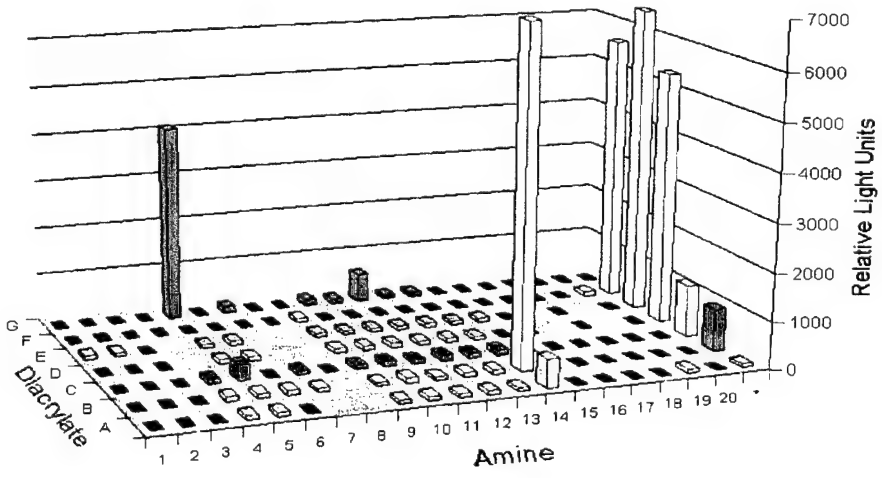


Figure 3: Transfection data as a function of structure for an assay employing pCMV-Luc (600 ng/well, DNA/polymer = 1:20). Light units are arbitrary and not normalized to total cell protein; experiments were performed in triplicate (error bars not shown). Black squares represent water-insoluble polymers, white squares represent water-soluble polymers that did not complex DNA in Figure 2. The right column (marked "*") displays values for the following control experiments: no polymer (green), PEI (red), and Lipofectamine (light blue).

The DNA-complexing materials identified in the above assay were further investigated in transfection assays employing plasmid DNA and the COS-7 cell line. All assays were performed simultaneously with the firefly luciferase reporter gene (pCMV-Luc) and levels of expressed protein were determined using a commercially available assay kit and a 96-well luminescence plate reader. Figure 3 displays data generated from an assay employing pCMV-Luc (600 ng/well) at DNA/poly ratios of 1:20 (w/w). The majority of the polymers screened did not

mediate transfection above a level typical of "naked" DNA (no polymer) controls under these conditions. However, several wells expressed higher levels of protein and the corresponding polymers were identified as "hits" in this assay. Polymers B14 (Mw = 3180) and G5 (Mw = 9170), for example, yielded transfection levels 4-8 times higher than control experiments employing poly(ethylene imine) (PEI), a polymer conventionally employed as a synthetic transfection vector, and transfection levels within or exceeding the range of expressed protein using Lipofectamine 2000, a leading commercially available lipid-based transfection vector system. Polymers A14, C5, G7, G10, and G12 were also identified as positive "hits" in the above experiment, but levels of gene expression were lower than B14 and G5.

Plan: The team will continue to evaluate the potential of this approach through the elucidation of structure/function relationships and the development of second-generation screening libraries.

Task 3: Transdermal Drug Delivery and Chemical Sensing for Neonates using Skin Electroporation
Principal Investigator: James C. Weaver, PhD, MIT

Our research into an improved method of transdermal drug delivery has yielded two major results. First, a new method for creating transdermal microconduits has been developed, which will be reported in a paper to be submitted for publication. Microconduits can be used for drug delivery and for interstitial fluid sampling. Second, a new method for carrying out function-based simulation of transport of charge, heat and molecules has been developed, and this will also be reported in a manuscript that is being written for submission to a scientific journal. The simulation has the potential to increase the productivity of research and development of minimally invasive technology, such as drug delivery protocols and devices that interface with tissue. This new, presently unpublished method was partially developed during our investigation of the skin's response to electrical pulses.

While investigating the optimization of microconduit creation in the skin's SC, the team has continued to seek understanding about how the multilamellar bilayer membranes within the SC are electroporated. This remains key to understanding how safe keratolytic agents are delivered into the SC, and how brief, highly localized heating takes place.

Key Results: During the past year new approach to computer simulation of spatially complex systems was identified. The team has obtained very encouraging results for a simulation of the transport of potent agents through the skin due to exposure of a small amount of the compound to the surface of the skin.

Specific Aim 1: Optimize parameters for creation of microconduits in hairless rat skin *in vitro*.

Specific Aim 2: Determine transdermal transport of aqueous solution through microconduits *in vivo* in hairless rats.

Specific Aim 3: Creation and partial optimization of microconduits in neonatal skin *in vitro*.

Progress: A new approach to computer simulation of spatially complex systems has been identified, with partial support from CIMIT. As noted previously, computer simulations have become a key part of the optimzation process, and have provided a partial insight and motivation

for creation of a new approach to computer simulations. The challenge is to understand how an applied voltage becomes rapidly redistributed within the spatially complex SC, as the SC is a system of irregularly shaped corneocytes surrounded by a spatially variable amount of lipid. The well established "brick wall" model of the SC is highly idealized. In this accepted model hydrated corneocytes are regarded as "bricks" and the surrounding multilamellar lipid bilayer membranes are viewed as "mortar". Our new simulation approach allows introduction of more realistic features, such as a stochastic spatial distribution of local voltage needed to cause electroporation. In addition to skin electroporation and microconduit formation, the new simulation method has the potential for broad application to many other problems in biomedical engineering. The team anticipates that the new simulation method will also allow important chemical transport problems to be solved. For example, the team has obtained very encouraging results for a simulation of the transport of potent agents through the skin due to exposure of a small amount of the compound to the surface of the skin.

Plan: Project completed. The team will continue to build on the successes to date, and evaluate both research and potential commercialization of the new microconduit method.

Task 4: Synthesize Vascularized Living Systems from the Platform of Two-Dimensional Silicon Microfabrication Technologies and Adapt to Three-Dimensional Living Devices
Principal Investigator: Joseph Vacanti, MD, MGH

The overall goal of this project is to develop tissue-engineered devices composed of living cells on matrices which, upon implantation, are vascularized either *in vitro* or *in vivo*. The major project related to this goal is to synthesize vascularized systems from the platform of 2-D silicon microfabrication technologies and adapt to three-dimensional living devices.

Successful vascularization of tissue engineered constructs using microfabrication requires the development of enabling new tools and techniques. During the past year, several critical processes were developed and tested. First, biocompatible polymer films of Polydimethylsiloxane (PDMS) were micropatterned and permanently bonded using rapid prototyping and soft lithographic methods. These devices were then used to establish techniques for culturing cells in microchannels, culminating in a successful four-week endothelial cell culture in a microfluidic circuit. In addition, processes for building biodegradable microfluidics were successfully established and tested. Feasibility of building mulitilayer three-dimensional microfluidic networks in PLGA 85:15 was demonstrated, and compatibility of bonding processes with porous films was established. These processes and techniques provide the necessary tools to build multilayer microfluidic devices in degradable polymers, seed them with combinations of parenchymal and nonparenchymal cells, and test hypotheses about vascularizing large tissue engineered constructs both *in vitro* and *in vivo*.

Key Results: Significant milestones in both fabrication and testing of microfabricated vascular scaffolds have been reached during this year. Foremost among these is fabrication of the latest vascularized network design, TEP-2, in both silicon micromachined and polymer cast scaffold materials. Initial fluid dynamic testing of these devices indicates that the twin goals of reduced pressure losses and more uniform flow have both been accomplished. Polymer fabrication in both biocompatible and biodegradable matrices is moving ahead swiftly. Molds produced from PolyDiMethylSiloxane (PDMS), a biocompatible polymer, have been produced in both two- and three-dimensions. Three-dimensional vascular beds have been connected in parallel and run

through initial fluid dynamic qualification studies. Bonded layers of biodegradable PLGA molded films have also been produced, a major milestone.

Specific Aim 1: Design and fabricate silicon and Pyrex based systems providing an array of etched channels to act as a mold for generating a living network in two dimensions. To accomplish this Specific Aim the following items will be addressed:

- Design and test systems to allow lifting and folding of the vascularized tissue from the etched silicon mold,
- Design bioreactors to house the device during tissue formation and folding,
- Develop assays to study the generation of tissue and its histological, biomechanical, and biochemical parameters,
- Investigate mechanisms of tissue development using molecular markers for gene developmental programs and programs of wound healing and regeneration, and
- Begin animal implantation studies to begin to understand perfusion, survival, and function of the living device.

Progress: Significant progress in the speed, reliability, and reproducibility of fabricating microfluidic devices was made during the past year. High-resolution photomasks were replaced by transparency photomasking for microfluidic applications allowing rapid prototyping, wherein a single researcher can design, print, micropattern a silicon mold, and create a new set of polymer devices within 1 day. In addition, once silicon molds are fabricated, they can be reused for casting indefinitely, requiring only 2 hours to build subsequent devices. Efforts to clamp etched wafers and flat substrates that proved unreliable in the past were replaced by plasma-assisted bonding of PDMS films to Pyrex wafers, significantly improving the reliability of sealed microfluidic devices and enabling reproducible cell culture without leaks or contamination (Figure 1). These processes were extended from two-dimensional microfluidic networks to interconnected three-dimensional networks using layer stacking and alignment (Figure 2). The processes together make up a robust tool-set for designing, fabricating, and testing cell culture in three-dimensional microfluidic scaffolds.

Figure **1** - *Topdown image of plasma-assited bonding of PDMS films to fabricate a microfluidic network.*

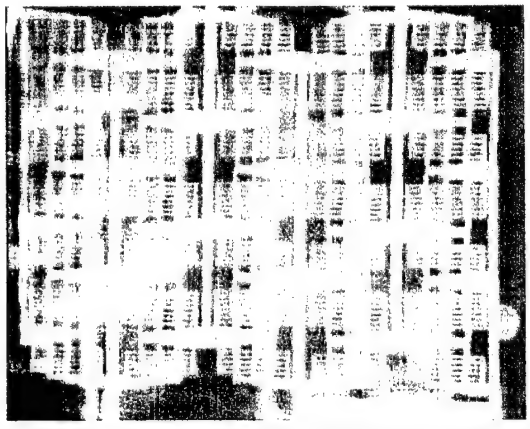


Figure **2** – *Top down image of multilayer bonded PDMS microfluidic network perfused with fluorescent dye.*

Microfluidic channels

Culturing cells in microfluidic channels is more challenging than in a standard petri dish due to the small fluid volumes, wall effects resulting from large surface area to volume ratios, and different forces governing motion (e.g. gravity becomes less important than surface tension in microfluidics). Therefore, new techniques are required for seeding and culturing cells in microdevices. During the past year several standard culture techniques were modified for microchannel cell culture and then demonstrated by long-term culture of endothelial cells in a simple microfluidic channel. Peristaltic flow pumps were replaced by syringe pumps to provide the extremely low flow rates required in microfluidic devices. Seeding cell concentrations were optimized to balance parasitic cell aggregation with desired high uniform seeding density. Appropriate culture flow rates were selected to deliver sufficient amounts of oxygenated culture medium while avoiding potentially harmful wall shear stresses. This work culminated in a demonstration of microchannel cell culture in which endothelial cells were cultured in a simple 100um x 30um cross-section, 5mm long microchannel for more than 4 weeks without contamination or leakage. (Figure 3)

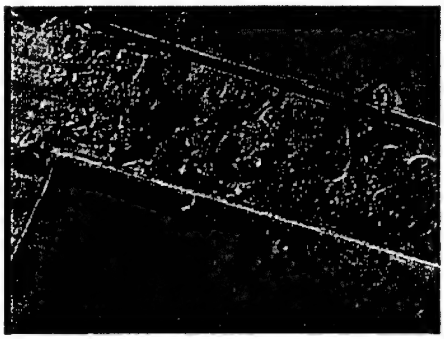


Figure **3** – *Top down image of rat lung endothelial cells in 100um x 30um microchannel. This culture was maintained for more than four weeks without contamination or leakage.*

Multi-layer scaffolds

The ultimate goal of this project is to build microfluidic scaffolds using biodegradable polymers for cell seeding and subsequent implantation. Therefore, during the past year, processes were developed to build fully biodegradable microfluidic devices. A two-step micromolding process was developed in which PLGA is melt processed or solvent cast on a flexible PDMS mold that had been previously molded from a microfabricated silicon mold with 2um resolution (Figure 4). Patterned films are then irreversibly bonded by temperature induced interdiffusion of polymer chains (Figure 6). This thermal fusion bonding process results in negligible pattern deformation (Figure 5), and allows for simultaneous bonding of multiple layers, thereby enabling rapid production of scalable three-dimensional designs (Figure 7). In addition, the processes are compatible with porous scaffolds as demonstrated by the permanent bonding of two fluidic channels with a porous layer interposed (Figure 8). Melt processing was selected as the optimal film fabrication process as it avoids the use of solvents, which cause film shrinkage and deformation of microstructure, and prevents potential toxic effects resulting from residual solvents. In addition, melt processing is fast, requiring only 1 hour to pattern and bond films compared to several days required for solvent processing. These processes were used to demonstrate the first fully biodegradable multi-layer microfluidic networks for cell culture and subsequent implantation.

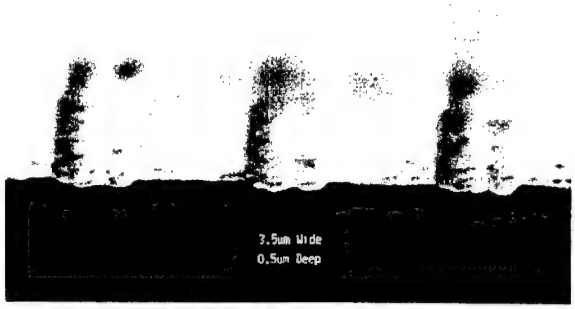


Figure **4** – SEM of 4um wide 0.5um deep channels patterned in PLGA

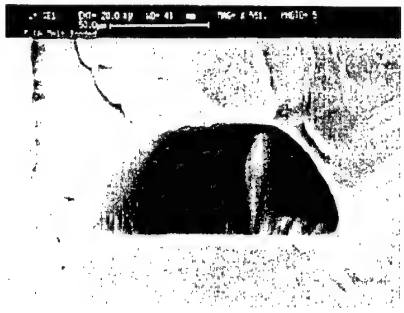


Figure 5 - SEM of 100 micron isotropic channel patterned PLGA bonded to a flat piece of PLGA.

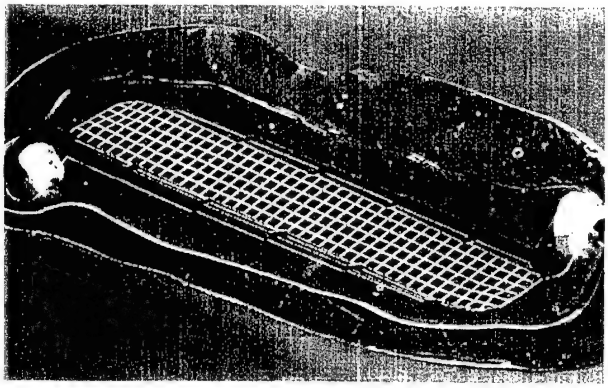


Figure **6** – Image of a fully biodegradable PLGA 85:15 microfluidic device with channels as small as 35um.

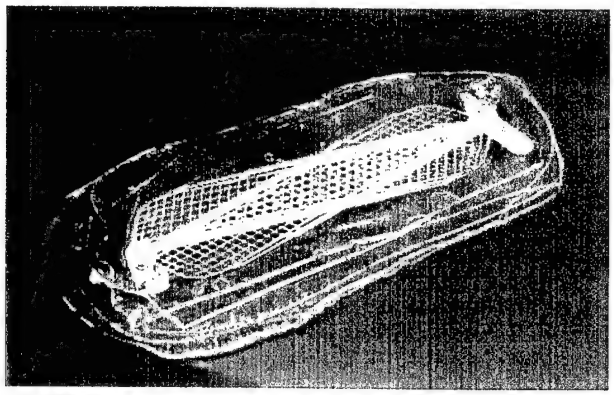
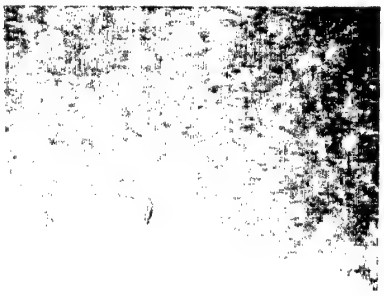


Figure **7** – Image of a interconnected multilayer fully biodegradable PLGA 85:15 microfluidic device with inlet and outlet connectors perfused with fluorescein dye.



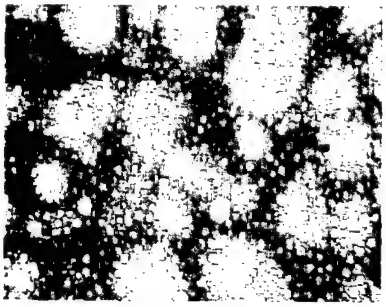


Figure **8** – A) Top down image of three layer structure. The stack consists of two microfluidic channels with an interposed porous film. Pore sizes are of order 1um and were not deformed during bonding. B) Close-up of pores after bonding.

Plan: Having developed the necessary tools and techniques for building polymer microfluidic networks during the last year, the team will shift the focus of our efforts toward understanding

cell growth in microfluidic devices. The team will compare the morphology, distribution, and growth kinetics of endothelial cells cultured in microchannels with those cultured in a standard petri dish. The goal of this future work is to understand cell growth and eventually direct cell growth in microchannels. When extended to co-culture, the ability to direct the growth of different cell types will enable development of function units of tissue. Using microfluidics, the diffusive transport challenges associated with scale-up can be overcome and massively parallel assemblies of functional units can be developed to constitute a functional organ.

Task 5: Synthesize Vascularized Living Systems from the Platform of Three-Dimensional Printing Technology

Principal Investigator: Joseph Vacanti, MD, MGH and Jeffrey Borenstein, PhD, Draper Laboratory, (DL), Cambridge, MA

Liver transplantation is the only established therapy for end-stage liver disease, however the shortage of donor organs has become a major limitation. For this reason, there has been great interest in cell transplantation as replacement therapy for enzyme deficiency disease or supportive therapy for acute and chronic hepatic failure. Using the principles of tissue engineering, the team has investigated the hepatocyte transplantation using polymer scaffolds as a means of generating new tissue replacement. The overall goal is to develop tissue-engineered devices composed of living cells on matrices which, upon implantation, are vascularized either *in vitro* or *in vivo*. The major project related to this goal is to synthesize vascularized systems from the platform of 3-D printing technologies.

Key Results: Major milestones in the design and microfabrication of scaffolds for endothelial cell seeding have been reached during the past year. In the area of design, several generations of networks have been produced, each representing a significant advance over previous designs. The first design with fully uniform flow, TEP-2, was generated on the wafer level and utilized to produce large numbers of silicon and polymer scaffolds for cell seeding. Design efforts were then transitioned to the modular networks TESTNET0 and TESTNET1, which are more suited to fluid dynamic experiments and biocompatibility studies. In a major advance, a new technique for generating mask layouts has been developed in which computational techniques are used to automatically produce vascular networks with desired flow characteristics. This new layout tool has been applied to the generation of photomasks for vascular designs, thereby reducing the cost of photomasks from $700 to $15 and the layout time required from 2 weeks to 1 day.

In the area of microfabrication, new process technologies based on micromolding have been developed and brought into routine practice for scaffold generation. High aspect ratio photoresist processes have been developed as a means to produce molds for scaffolds as an alternative to advanced silicon etching technology. Controlled sidewall profile silicon etch processes have also been developed as a means to produce scaffolds which are optimized for cell attachment and expansion. Large numbers of multi-layer networks have been produced in biocompatible polymeric materials, using micromachined through-hole technology. For the first time, a high-resolution microfabricated biodegradable device has been produced, and the process used to build it is currently being extended to produce the first fully biodegradable three-dimensional scaffolds for the microcirculation.

Specific Aim 1: Design and fabricate silicon and Pyrex based systems providing an array of etched channels to act as a mold for generating a living network in two dimensions. To accomplish this Specific Aim the following items will be addressed:

- Design and test systems to allow lifting and folding of the vascularized tissue from the etched silicon mold,
- Design bioreactors to house the device during tissue formation and folding,
- Develop assays to study the generation of tissue and its histologic, biomechanical, and biochemical parameters,
- Investigate mechanisms of tissue development using molecular markers for gene developmental programs and programs of wound healing and regeneration, and
- Begin animal implantation studies to begin to understand perfusion, survival, and function of the living device, and
- Design and test systems to allow lifting and folding of the vascularized tissue from the etched silicon mold.

Progress:

Uniform Flow Wafer-Level Designs

A major milestone was reached with the implementation of a new design for the vascular branching network. This design, shown in Figure 1, is based upon a fluid dynamic model which provides uniform flow to all branches of the network. Scaling laws for the channel dimensions are based upon morphometric data from the vascular physiology. Two-dimensional microfabrication technology has also been advanced during the past year. Early designs were fabricated using a single depth for all channels, from the widest veins and arteries down to the narrowest capillaries. New micromachining processes, based upon specialized photolithography and sequential plasma etching steps, produce deeper channels to emulate the wider vessels, resulting in the structure shown in Figure 2.

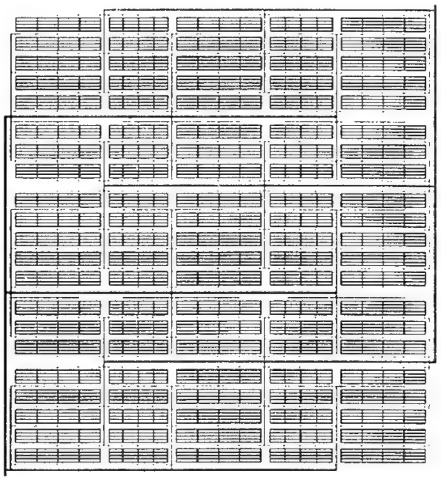


Figure 1. *TEP-2 wafer-level network, which is modeled based on morphometric data obtained from animal physiology. This was the first network to demonstrate completely uniform flow in laboratory fluid dynamic studies.*

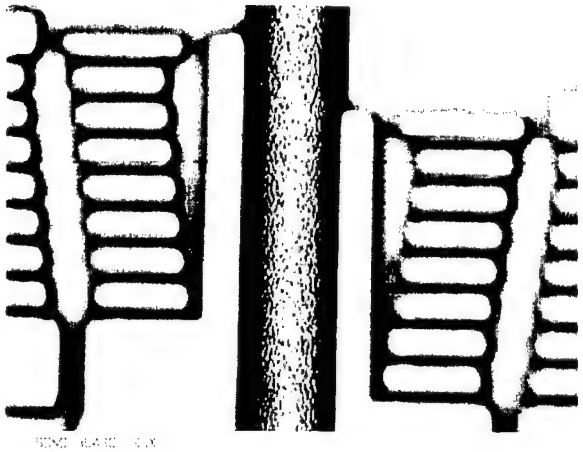


Figure 2. *TEP-2 wafer-level network, micromachined in silicon using a two-depth etching process. Note the wide central channel, an arterial scaffold, juxtaposed next to much shallower capillaries.*

Two-Depth Silicon Micromachining Processes

Initial fabrication results on this new device have been excellent. Unlike TEP-1, this design requires two different channel depths within the wafer mold, which presents a challenge for the silicon microfabrication process. A new process for building two-level designs has been developed and demonstrated. Shallow channels, such as the capillaries and the first-level arterioles which feed them, have been etched to a depth of 25 microns, resulting in nearly circular cross-sections. Deeper channels, such as the sewable input and output vessels and the branching arteries and veins, have been etched to a depth of 200 microns, an accomplishment made possible by state-of-the-art micromachining technology recently developed at Draper Laboratory. Measurements of pressure drops in the TEP-2 networks demonstrate that physiological levels of pressure result in very rapid and uniform flow through the matrix. Microfluidic analysis using round- and square-bottomed channels provide excellent agreement with experimental data.

New Low-Cost, Rapid Prototyping Design Capability

Cell culture work by the Vacanti Laboratory at MGH has provided important feedback to the microfabrication effort in a number of critical areas. Two of the most significant requirements of the cell culturing activity are to obtain scaffolds with new designs quickly, and in an overall form factor which supports biocompatibility studies. During this second half of the past year's program, efforts have been shifted to rapid prototyping of a series of new vascular network designs for a variety of tissues and applications. The primary goal is to build a capability to customize networks for specific applications such as liver, kidney, nerve and other organs. Key design parameters include targeted blood flow rates, velocities, pressure drops and shear levels. The new designs are produced in a semi-automated fashion by entering targeted fluid dynamic properties into a MATLAB® program, which computes a network which meets the user's criteria. The program generates a file of a special type, which is read into a standard photomask layout editor for final modifications and integration with world-to-chip connections.

Photomask layout generation has been transferred from a complex, workstation-based process, which typically takes two weeks to complete, to a rapid-turnaround, desktop-based software package which provides a completed file in less than a day. A new process for generating the actual photomasks has been implemented, in which the new layout tool sends an output file to a high-resolution transparency printer. The cost of this new process, $15 per mask, compares favorably with that of the previous method ($700), where an electron-beam writer produces a high-resolution print on a quartz plate. Only one drawback of the new technique exists; the

resolution limit of the printing method is 30 microns rather than the 0.1 micron resolution obtained with the electron-beam technique. Therefore, initial work will utilize the new technique, while projects requiring capillaries of 10 micron diameter will continue to use the original layout and photomask generation process. An example of a vascular network designed using the new computation tool is shown in Figure 3, below.

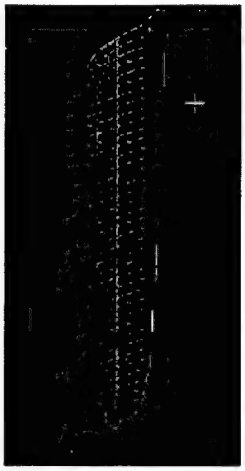


Figure 3. *New capillary design, TESTNET0, which was generated using a computational tool which provides uniform flow, physiological values for pressure drops and maximum shear, and desired fluid velocities in the channels. Current efforts are focused on integrating the analysis of Dr. Kaazempur-Mofrad into this model, in order to quantify the effects of non-Newtonian blood rheology and the mixed-phase nature of blood due to the presence of hematocrit.*

Plan: In the next year of work, the design effort will continue to utilize the rapid prototyping method reported here. Complex fluid mechanical phenomena, such as non-Newtonian rheology, hematocrit, and channel distensibility, will be integrated into the modeling and design process, so that scaffolds replicate the physiology as closely as possible. Three-dimensional design and analytical capabilities will also be developed.

Silicon microfabrication technology will continue to play a critical role in defining and shaping the three-dimensional polymer scaffolds which are cast from the molds. Substantial efforts will be focused upon the development of specialized silicon etching techniques which produce controlled sidewall profiles for optimization of cell seeding and attachment. Such techniques will be aimed at producing scaffolds with smooth, rounded profiles which promote the generation of confluent layers of tissue.

Biocompatible Polymer Membranes and 3D Networks

The importance of translating silicon micromachining onto biodegradable polymers, in order to extend the vascularized tissue engineering technology into the third dimension, was recognized at the outset of this program. During the past year, significant progress in this arena has been achieved. Micromachined wafers are routinely fabricated to produce a positive mold, in which the vascular channels are etched into the wafer surface, or a negative mold, in which channels are raised above the background. In addition, polymeric molds have been produced using advanced High Aspect Ratio Structures (HARS) such as epoxy resin photolithography and Polydimethylsiloxane (PDMS) micromolding. Figure 4, below, illustrates the use of HARS technology to produce thick (200 micron) films of polymeric material with high precision (< 10 micron) channel dimensions. Such wafers can be used as a mold to emboss additional polymer films. At the right, in Figure 5, is a frame captured from a microfluidic flow study in a closed vessel network. Fluorescent beads in the size range of red blood cells have been inserted into the network, which is not visible under fluorescent light. Note that the rapid motion of beads (blurred images) simulates the traversal of red blood vessels through the narrow capillaries.

The epoxy resin, SU-8, is capable of building HARS as tall as 300 microns. For the TEP-2 design, two-level HARS have been produced in SU-8 epoxy, with one level at 25 microns and the other at 200 microns in depth. This two-level fluidic interconnect structure is required for uniform flow in the TEP-2 design, and represents a major advance in fabrication capability. From the epoxy mold, PDMS polymer scaffolds have been cast and sealed. Figure 6, below, shows an image of a two-level PDMS cast from the TEP-2 design. At the lower right is the fluid input port, which can be sewn in to the artery as the entry into the scaffold network once the device has been seeded. At the upper right is the output port, which represents the venous outflow. Figure 7 shows fluorescent dye filling the two-level PDMS scaffold; the flow is extremely uniform and occurs without gaps or bubbles. Fluid transfer across the shallow-to-deep transition between capillaries and larger vessels occurs without turbulence or dead space, an excellent result Processes described so far utilize materials which are not biodegradable; however, they can serve as a reusable template for production of biodegradable polymer films. Formation of closed vessels is now being accomplished by plasma-assisted polymer bonding processes. Release of the polymer scaffolds from silicon micromachined molds is enabled by the introduction of a newly-invented plasma deposition process.

Through-hole technology to connect two-dimensional vascular beds has been developed and is working well, as evidenced by this two-level system. Two channel layers are first produced using standard replica molding techniques. In between the two channel layers, a layer with through-holes is sandwiched and the triple stack is bonded. The major challenge is to produce through-holes which form a continuous open path from the lower channel network to the upper network. Replica molding is also used to produce the through-hole layer, and a controlled clamping pressure is applied to the film in order to produce a series of continuous through-holes from the bottom to the top of the film. This new method replaces the syringe puncture process previously used to demonstrate three-dimensional capability. Replica molding of through-holes is a manufacturable process which could be extended to stack tens or hundreds of layers together, depending upon the size of the target organ. Development of this process represents a critical milestone in the effort to scale up the microfabricated tissue engineering technology.

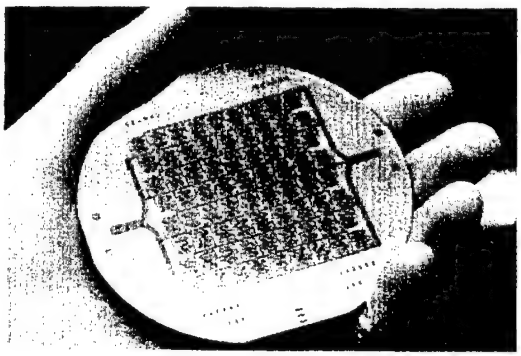


Figure 4. HARS polymer film deposited and patterned on silicon wafer.

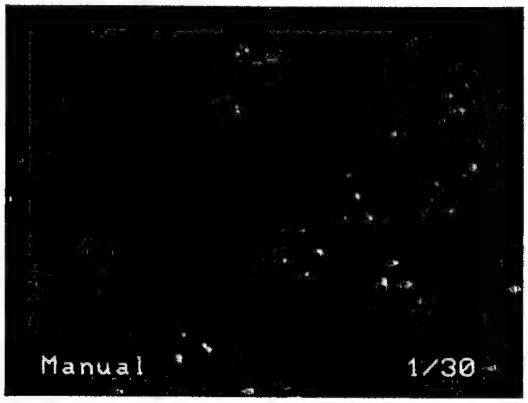


Figure 5. Microfluidic study using fluorescent beads in narrow channel flow.

Figure 6. Dual-Layer Polymer Film Cast with TEP-2 Design.

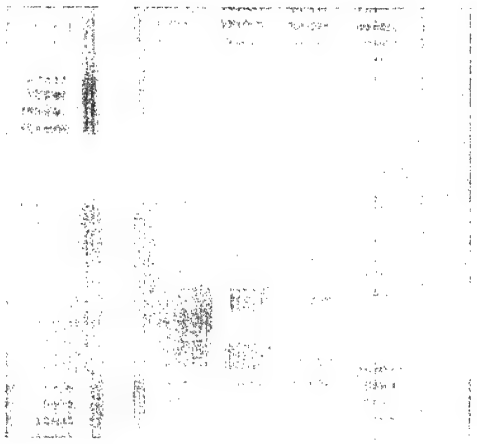


Figure 7. Fluorescent dye-filled TEP-2 Vascular Scaffold.

Fluid Dynamic Characterization

Substantial efforts have been focused on the experimental characterization of these new networks. Experiments involving fluorescence-labeled beads and red blood cells immersed in a carrier fluid are now underway, with the goal of generating data which can be compared with models developed by Eli Weinberg and by Mohammad Kazeempur-Mofrad. Dr. Mofrad has been running analyses of each of the new designs in order to fully characterize the distribution of flow and pressure, the wall shear stress and the influence of a mixed-phase system (containing hematocrit) on the fluid dynamics. Eli Weinberg has been measuring the pressure vs. flow curves for each of the designs in the PDMS channels built by Kevin King and Joanne Wang, and has addressed some of the key issues which will determine the overall performance of these systems in the bioreactor. One such key parameter is the distensibility of the system. Venous distensibility is important in the regulation of blood pressure and the distribution of blood flow in animal physiology, but has not been formally addressed in a MEMS-based system because traditional platforms such as silicon and glass are rigid. However, the new polymer MEMS technology being developed for this program utilizes polymers which are flexible and have a low Young's modulus, and are therefore susceptible to distensibility effects. In Figure 8 below, gauge pressure vs. flow data is shown for two values of ambient pressure: atm + 0 mm Hg and atm + 100 mm Hg. The marked increase in flow for a given pressure indicates that the distensibility of the PDMS network must be accounted for when designing a microfluidic system for cell culture.

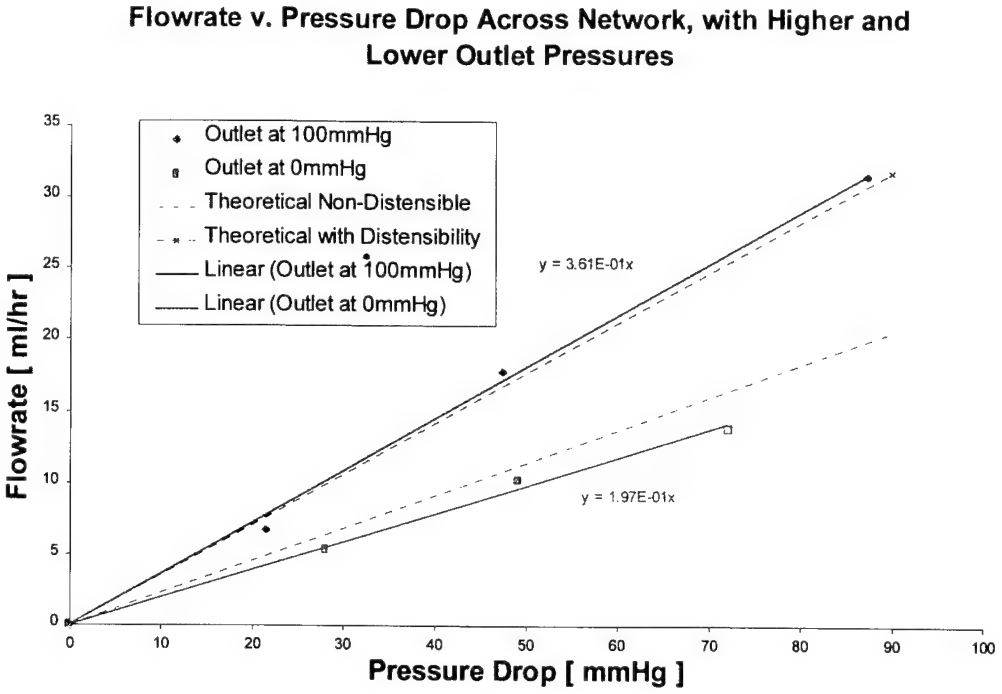


Figure 8. Distensibility measurements for TESTNET0 geometry, for 2D PDMS mold.

High-Resolution Biodegradable Microdevice Fabrication

For the first time, biodegradable scaffolds have been produced with very high (2 micron) resolution, and have been bonded together with no intervening adhesive layers. This represents a major milestone in the program, and moves the effort closer to the realization of a 3-dimensional.

biodegradable endothelialized network which may be implanted for animal studies. The process, developed by Kevin King and Joanne Wang, utilizes a two-step micromolding process, in which PDMS molds are cast on silicon and then used to cast PLGA molds. While the smallest structures on the tissue engineering constructs are on the order of 30 microns in diameter, higher resolution is desired in order to build smooth, well-controlled sidewalls which are optimized for cell attachment and growth. A test mask with high-resolution features as small as 2 microns was utilized to test the ultimate resolution of the biodegradable polymer process, and 2 micron lines and spaces were produced with relative ease. An image of the 4-micron features reproduced from a high-resolution mask is shown in Figure 9, below.

For the closed channel networks, a bonding process for the PLGA constructs is required. Such a process has been developed and demonstrated, and a multilayer scaffold has been produced. This device is shown in Figure 10. No intervening adhesive layer is required, making this the first fully degradable high-resolution microdevice ever produced.

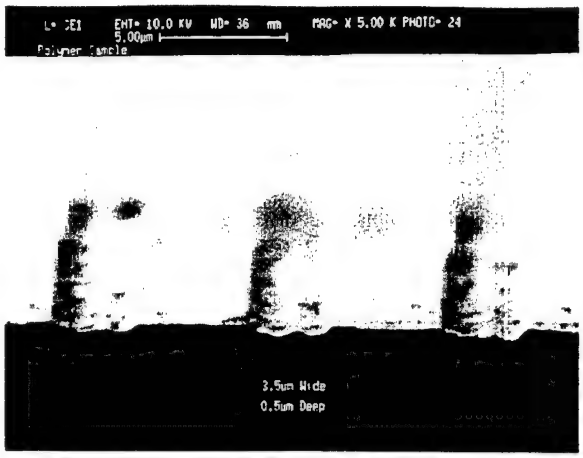


Figure 9. Scanning electron micrograph of the 2-micron lines and spaces replicated on a PLGA platform.

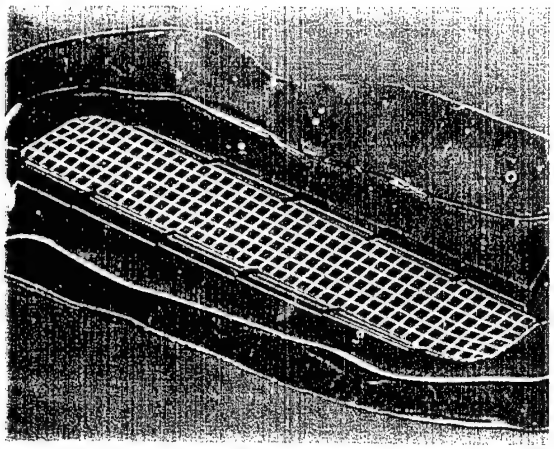


Figure 10. Fully degradable high-resolution microdevice built by casting PLGA on a PDMS master mold.

Plan: In the next year of work, efforts will be focused on continuing the development of the 3 dimensional microdevice for cell culturing in both the PDMS biocompatible and PLGA biodegradable platforms. In addition, technology developed at the laboratory of Professor Robert Langer of MIT will be utilized to demonstrate high-resolution microfabrication of additional biodegradable polymers. Finally, techniques to provide microporous cast films and membranes will be explored, for integration into 3D microdevices in which endothelial and parenchymal cells will be co-cultured.

Task 6: Minimally Invasive Meniscal Repair with Tissue Engineered Cartilage
Principal Investigator: Thomas J. Gill, MD and David J. Zaleske, MD, MGH

Recent findings demonstrate that a cell-based therapy could be one therapeutic approach for repairing tears in the avascular zone of the knee meniscus cartilage. In previous studies in the nude mouse model, articular chondrocytes seeded onto a devitalized meniscal cartilage scaffold were able to bond the edges of a meniscal tear. Those studies were performed in a vascular subcutaneous environment, which is different from the avascular articular environment of the knee joint. The studies performed over the past year demonstrated that the same goal could be achieved in the articular environment in a large animal model. The results of the first year of investigation demonstrated that this tissue engineered approach could repair the knee meniscus when a lesion involves the avascular inner third. Although further investigation is still needed to define the best delivery material and the best pre-seeding conditions of the reparative cells onto such a scaffold, the team believes that a clinically applicable approach combining this technique with arthroscopic surgery might be soon developed.

The aims of this grant are: 1) to demonstrate that chondrocytes, seeded onto a matrix scaffold, can be used as valid therapeutic approach to achieve a secure meniscus repair in a preclinical orthotopic model; and 2) to investigate other cell sources and different absorbable materials for cell scaffolding to accomplish a minimally invasive meniscus repair technique. In previous studies in the nude mouse model, articular chondrocytes seeded onto a devitalized meniscal cartilage scaffold were able to bond the edges of a meniscal tear. Results from these studies demonstrate that a cell-based therapy could be a useful therapeutic approach for repairing tears in the avascular zone of the knee meniscus cartilage.

Key Results: Studies performed over the past year demonstrated that a cell-based therapeutic approach can be used in the articular environment in a large animal model of meniscal tears. Further investigation during the second year sought to define the best delivery material and the best pre-seeding conditions of the reparative cells onto candidate scaffolds. A clinically applicable approach combining this technique with arthroscopic surgery might be developed based on these studies.

Specific Aim 1: To demonstrate that chondrocytes, seeded onto a matrix scaffold, can be used as valid therapeutic approach to achieve a secure meniscus repair in a preclinical orthotopic model.

Rationale: During the past year, the team has demonstrated meniscal healing in a nude mouse model, using articular cartilage chondrocytes as the cells source and devitalized meniscal chips as structural support for chondrocytes. The central hypothesis of this project is that a lesion in the meniscus can be repaired using isolated autologous cells seeded onto a scaffold material. The scaffold could be allogeneic devitalized meniscal tissue or other synthetic materials to be investigated. The cells could be chondrocytes or other cell population, stimulated to chondrogeneic differentiation. The cell-seeded construct would be then interposed in the meniscal lesion and secured in place. Healing would be achieved by the bonding capabilities of the cells. The goal of this section will be to develop and refine the model for creating a reproducible meniscal injury in the medial meniscus of pigs. Once this goal is achieved, new

constructs or variables to be investigated in subsequent stages of the project will be tested in the same fashion for consistency.

Progress: This past year, nineteen pigs were operated in which a bucket-handle lesion was made in the medial meniscus of the left knee (Table 1). In the six animals in group A, the lesion was treated with a scaffold seeded with autologous articular chondrocytes and secured into the lesion with a suture. In four of those, the scaffold material was a devitalized allogeneic meniscal cartilage (group A1), whereas in the last two, the cells were delivered by a collagen sponge (group A2). In the five animals in control group B, the lesion was treated with the scaffold without seeded cells and secured with a suture. In four of those, the scaffold material was a devitalized allogeneic meniscal cartilage (group B1), whereas in the last case, an unseeded collagen sponge was utilized as control (group B2). Four animals in control group C had the lesion treated with the simple suture. The lesion was left untreated in four animals in control group D.

Table 1

Pig #	Date 1st surgery	Side	Date 2nd surgery	Lesion/Treatment	Follow-up	Date Sacrcifice	General Results
18	1/5/00	L	--	Suture only	9 weeks	3/8/00	Not repaired
19	1/5/00	L	--	Scaffold w/o cells	9 weeks	3/8/00	Not repaired
20	1/18/00	L	2/9/00	Exp: scaffold w/cells	9 weeks	4/12/00	Weak repair; chip in site
21	2/8/00	L	2/24/00	Exp: scaffold w/cells	9 weeks	4/24/00	Weak repair; chip in site
22	2/8/00	L	2/24/00	Exp: scaffold w/cells	9 weeks	4/24/00	Weak repair; chip in site
644	6/6/00	L	--	Scaffold w/o cells	9 weeks	8/8/00	Not repaired
643	6/6/00	L	--	Untreated	9 weeks	8/8/00	Not repaired
23	7/6/00	L	--	Suture only	9 weeks	9/7/00	Not repaired
24	7/6/00	L	--	Untreated	9 weeks	9/7/00	Not repaired
656	7/13/00	L	--	Scaffold w/o cells	9 weeks	9/11/00	Not repaired; chip in site
657	7/13/00	L	--	Suture only	9 weeks	9/11/00	Not repaired
658	7/13/00	L	--	Suture only	9 weeks	9/12/00	Not repaired
659	7/13/00	L	--	Untreated	9 weeks	9/12/00	Not repaired
660	7/18/00	L	8/3/00	Exp: sponge w/cells	9 weeks	10/3/00	Not repaired
661	7/18/00	L	8/1/00	Exp: scaffold w/cells	9 weeks	10/3/00	Not repaired
662	7/21/00	L		Untreated	9 weeks	9/21/00	Not repaired
663	7/21/00	L		Scaffold w/o cells	9 weeks	9/21/00	Not repaired
666	9/21/00	L		Sponge w/o cells	9 weeks	9/27/00	Not repaired
667	9/21/00	R-L	10/6/00	Exp: sponge w/cells	9 weeks	12/11/00	Some sporadic bonding

Gross evaluation showed bonding of lesion margins in three specimens out of four from group A1 (Figure 1A). Poor and incomplete repair was seen, on the other hand, in the two specimens of group A2, treated with a cell-seeded collagen sponge. Histological analysis of the sponges, seeded with the chondrocytes but not utilized for meniscal repair surgery, showed a non-uniform distribution of the cells within the collagen web, indicating that still further work is needed in the seeding process. Macroscopic analysis of the all control specimens indicated the presence of not repaired lesions.

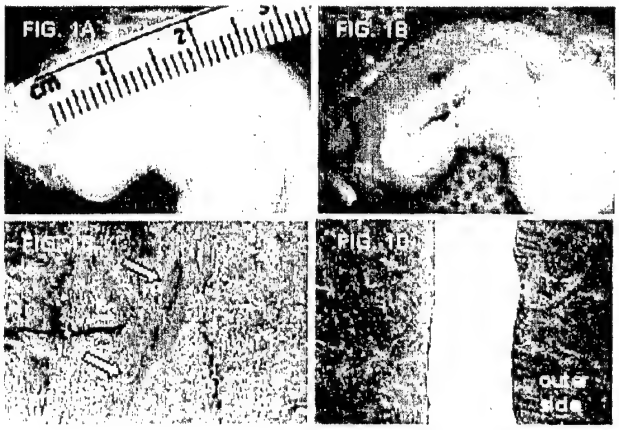


Figure 1. Histological analysis shows complete adherence between the margins of meniscal fracture and the cell-seeded scaffold in several areas in the specimens of group A1 menisci (Figure 1C); the arrows in the picture 1C represent the limit between the scaffold (left) and the outer part of the meniscus. Other areas of the same specimens showed interruption of continuity between the seeded scaffold and the native meniscus lesion edges. Where repair was achieved, newly formed cartilage matrix was involved in the bonding process. On the other hand, no matrix formation nor signs of repair was seen in the specimens of all control groups (Figure 1D).

Plan: Project completed. A manuscript on these results is completed and submitted to Journal of Orthopedic Research.

Specific Aim 2: To investigate other cell sources and different absorbable materials for cell scaffolding to accomplish a minimally invasive meniscus repair technique.

Cells

Although autologous chondrocytes may serve as the 'gold standard' for assessing the repair ability in the early phases of these studies, the morbidity associated with harvesting articular chondrocytes from uninvolved joints may preclude clinical application of these techniques. Alternate sources of cells may be adequate or even preferred over the use of articular chondrocytes. In year 2, studies using the following cell types were performed:

1. Articular chondrocytes (Figure 2)
2. Bone marrow stem cells (Figure 3)
3. Synovial cells (Figure 4)

4. Dermal fibroblasts (Figure 5)
5. Auricular chondrocytes

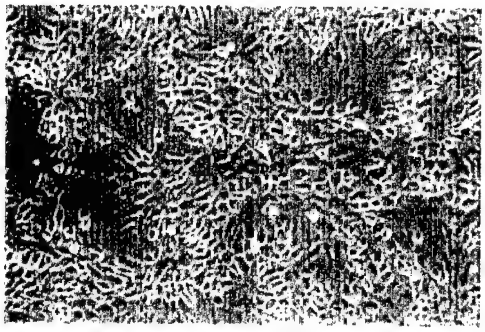


Figure 2. Chondrocytes

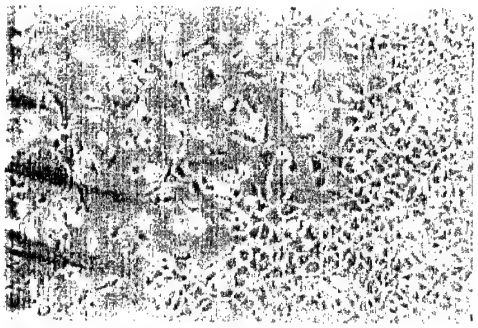


Figure 3. Bone Marrow Cells

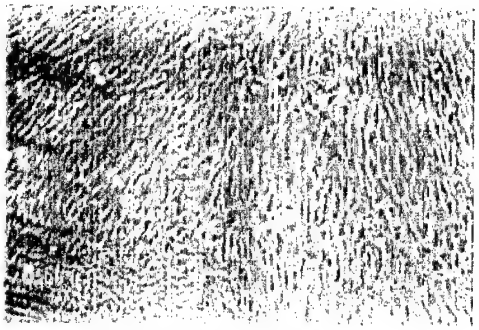


Figure 4. Synoviocytes

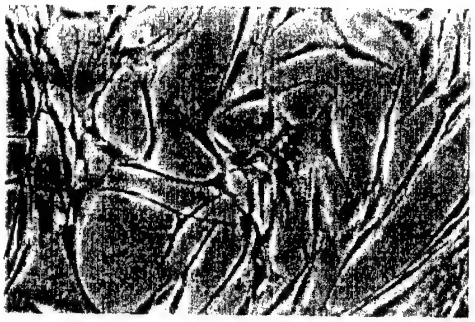


Figure 5. Dermal Fibroblasts

Progress:

Cell Sources

Articular chondrocytes have been employed in the orthotopic swine model under Aim 1. Those results demonstrated that partial bonding of a meniscal lesion can be achieved using articular chondrocytes. Progress under this task included isolating bone marrow cells, dermal fibroblasts, and synovial cells for as candidate cell sources for use in meniscal repair.

Bone marrow cells were isolated from the pig bone marrow, dermal fibroblasts were isolated from pig skin, and synovial cells were harvested from the knees of pigs cells by enzymatic digestion. All cell types have been grown in monolayer culture to confluence. All three types of cells were cultured for 1-2 passages and combined with periosteum for repairing meniscus lesion to test these cells for their ability to form bonds in meniscal tissues in the nude mouse model.

Cultured chondrocytes (Figure 6a), synoviocytes (Figure 6b), and bone marrow cells (Figure 6c) have been used in conjunction with periosteum to repair a bucket handle lesion in a meniscus placed in the backs of nude mice. Periosteum alone was used as the control (Figure 6d). The results showed that all meniscal lesions healed. When chondrocytes are used, however, there is notable production of new cartilage matrix.

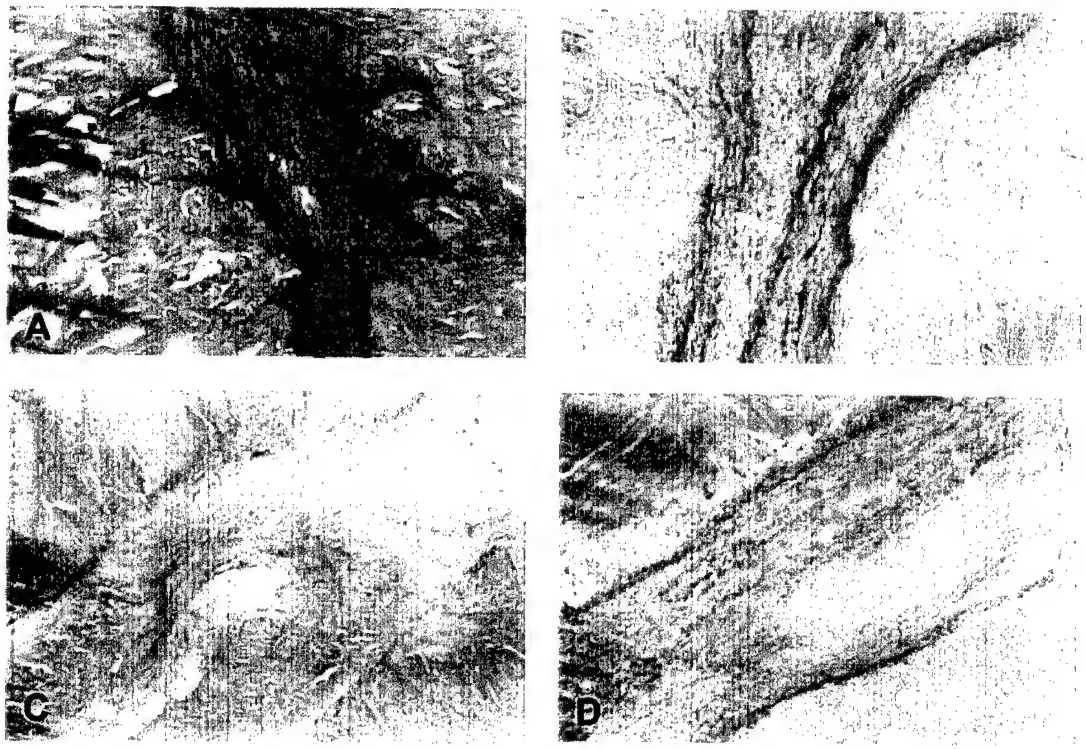


Figure 6. Meniscal repair using periosteum bonded with chondrocytes (a), synoviocytes (b), bone marrow cells (c), and control without cells (d).

Plan: Biomechanical testing on the healing using these approaches is being performed. The results are being analyzed and incorporated into manuscripts.

Scaffolds

The scaffold employed in the orthotopic swine study was meniscal tissue, devitalized through freezing/thawing cycles. However, this method presents technical problems in a clinical setting. If autologous tissue is considered, availability of suitable scaffold would be the limiting factor. If allogeneic meniscus is employed, issues related to safety of the implant must be considered. Using alternative autologous tissue for a scaffold or synthetic bioresorbable materials could be more desirable. The goal of this section was to test the following new scaffold materials.

1. Polyglycolic/polylatic acid mesh
1. Resorbable PLGA foams
2. Collagen foams
3. Perichondrium
4. Periosteum (data above in section on cells)
5. Mixed biological/synthetic materials

Resorbable Polyglycolic/polylatic acid mesh (Vicryl®) with Auricular Chondrocytes

Fibrin glue is a biological polymer, which may be an ideal scaffold for holding reparative cells. However, its gel-like property could limit clinical application. The addition of Vicryl mesh to fibrin glue/cell composite could contribute to stabilize the implant before the transplanted cells have started to produce the reparative matrix.

Devitalized swine menisci were used as repair model simulating the avascular portion of meniscus. A 1 cm lesion was made in devitalized swine meniscus. Samples were divided in three groups: 1) lesion treated with knitted Vicryl mesh-auricular chondrocytes-fibrin glue (Group 1); 2) lesion treated with woven Vicryl mesh-auricular chondrocytes–fibrin glue (Group 2); and 3) lesion left untreated (control group, Group 3). As the lesion was treated, all samples were wrapped in fibrin glue (without cells) to simulate diffusion conditions in the knee joint. Samples were implanted into nude mice for 8 weeks. Some samples were processed for histological evaluation. In others, gross mechanical testing was performed.

Histology revealed that neocartilaginous matrix formed in both knitted and woven Vicryl mesh groups. A tight bonding was formed between meniscus lesion and matrix. Vicryl mesh was still present in woven Vicryl mesh group (Figure 7-B), whereas Vicryl mesh could not be found in knitted Vicryl mesh group (Figure 7-A). No evidence of healing occurred in the control group (Figure 7-C). Gross mechanical testing showed weak healing in knitted and woven Vicryl mesh groups, whereas no healing in control group. It is possible to use Vicryl mesh-auricular chondrocytes-fibrin glue as part of a composite for meniscus repair. Knitted Vicryl mesh might be preferable because of its short term of absorption.

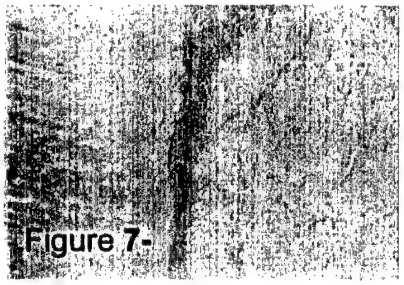
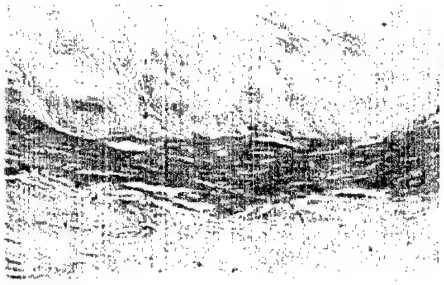


Figure 7-



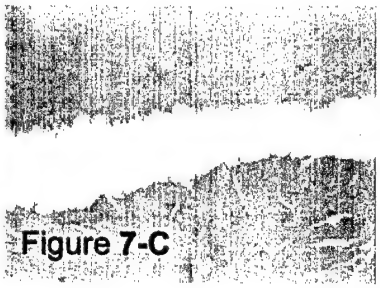


Figure 7-C

Perichondrium Scaffold

Perichondrium can be used as both scaffold and a source of cells to generate neo-cartilage and facilitate repair. The use of perichondrium for generating cartilaginous matrix to repair a lesion in meniscus using the nude mouse model was performed.

The following six experimental groups were designed: 1) whole thickness perichondrium graft/ fibrin glue/auricular chondrocytes (Group 4, see Figure 8, n=10), 2) split thickness perichondrium graft/ fibrin glue/auricular chondrocytes (Group 5, see Figure 9, n=10), 3) folded perichondrium (cambium layer out and fibrous layer bonded with Dermabond Glue)/ fibrin glue/ chondrocytes (Group 8, see Figure 10, n=10), 4) whole thickness perichondrium graft (Group 7, n=10), 5) split thickness perichondrium graft (Group 8, n=10), and 6) folded perichondrium graft (Group 9, n=10). All the constructs were implanted into dorsal subcutaneous pocket in nude mice for 8 weeks.

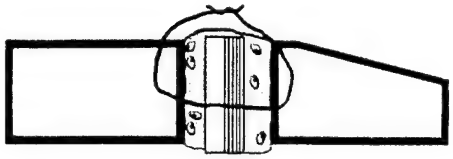


Figure 8. Experimental design of Group 4

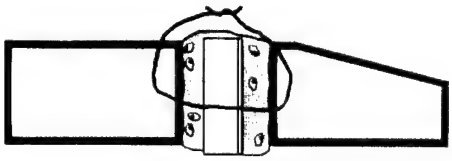


Figure 9. Experimental design of Group 5

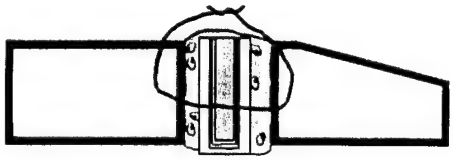


Figure 10. Experimental design of Group 8

There was no significant difference between the healing rates of Group 4 (whole thickness perichondrium, Figure 11) and Group 5 (split thickness of perichondrium, Figure 12).

Figure 11.

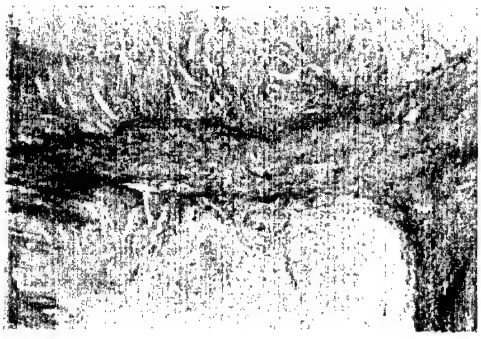
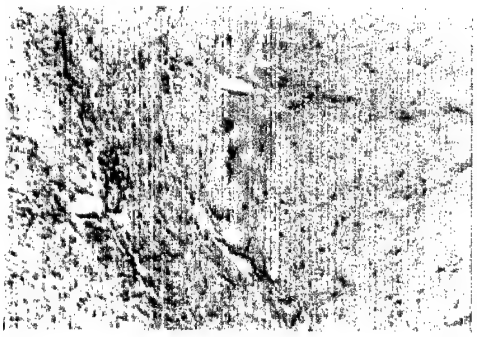


Figure 12.



Plan: Although bucket-handle tears in devitalized meniscal could be healed by both knitted and woven Vicryl mesh combined with auricular chondrocytes and fibrin glue, the finial decision of choosing which kind of Vicryl mesh would rely on the biomechanical testing to be performed during the next quarter. Perichondrium is useful as a source of cells as well as a scaffold. Results are favorable in healing a meniscal lesion in the nude mouse model. These results are being written into a draft manuscript.

Task 7: Development of a Novel *in vivo* Recombinant Protein Delivery Device Designed to Regress Abnormal Tissue: Recombinant Human Müllerian Inhibiting Substance (rhMIS) Producing Cells on Biodegradable Matrices
Principal Investigator: David T. MacLaughlin, PhD, MGH

A significant problem impeding progress in using complex human proteins as therapeutic agents against disease is producing sufficient quantities for use in a cost-effective manner and the

development of a suitable and effective system to deliver these proteins to patients. These proteins which include growth factor, hormones with inhibiting activities will be used in a variety of settings including as replacements for absent proteins and as treatments for cancers in others. Most of these proteins are complex macromolecules consisting of subunits and/or covalently attached substituent groups. In addition, they are produced in extremely small quantities naturally and it is impossible to purify enough for use in the clinic. Hence, there are significant problems to overcome before the cost-effective production of sufficient quantities of highly purified proteins as drugs can be accomplished. Modern cloning strategies have been used to create organisms to produce large quantities of the proteins but despite this tremendous technological breakthrough, elaborate purification strategies are still needed to prepare the products for clinical use. These methods are expensive and the processes have modest yields with reduced biological potency of the products. Our project is designed to eliminate both the requirement for *in vitro* production facilities and the need to purify the proteins. The team used Müllerian Inhibiting Substance (MIS) as a model protein and the treatment of ovarian cancers *in vivo* as a biological assay to test the hypothesis that bio-engineered implantable tissue could be used as a drug delivery system.

The team has also begun to permanently transfect human and other species fibroblast cell lines with a construct of the human MIS cDNA that is nested with a gene for antibiotic resistance. A mouse line, 3T3, has incorporated the cDNA and 24 sub-clones, are currently being maintained under antibiotic selection. A human line, IMR 90, has also been transfected and, when sufficient numbers of cells are obtained, these will be sub-cultured as well as will the non-cancer human fibroblast line BJ, which has also been transfected with the MIS gene *via* electroporation protocols.

Key Results: During the past year, resorbable polyglycolic acid biopolymer matrices impregnated with cells transfected with the MIS gene were successfully implanted in over 80 immuno-compromised mice and bioactive MIS produced and absorbed by the blood stream. The effect of different sized biopolymer implants on the resulting serum MIS concentrations was also determined. There is a size-dependent accumulation of MIS in the serum of animals particularly after 10 days to 2 weeks incubation (partial completion of Specific Aim 1). Furthermore, MIS delivered to animals by the cell seeded biopolymer inhibited the growth of human ovarian cancer cell xenographs placed in the subrenal capsule (partial completion of Specific Aim 2) relative to control grafts seeded with cells expressing a mutant MIS devoid of bioactivity or the empty polymer. A manuscript describing these results was published in the Proceedings of the National Academy of Sciences (Stephen AE, Masiakos PT, Segev DL, Vacanti JP, Donahoe PK, MacLaughlin DT. Tissue-engineered cells producing complex recombinant proteins inhibit ovarian cancer *in vivo*. Proc Natl Acad Sci U S A. 2001 Mar 13;98(6):3214-9) and a provisional patent application related to this subject (U.S.S.N. 60/178,842 "Delivery of Therapeutic Biologicals from Implantable Tissue Matrices" Vacanti et al,) was filed in January.

Specific Aim 1: Determine *in vivo* pharmacokinetics of recombinant human MIS produced on degradable biopolymer matrices implanted into SCID mice by A) transfected clonal CHO B9 cells and B) transfected or virally infected murine and human fibroblast cell lines.

Progress: The growth of MIS gene-transfected cells (CHO-B9) on the biopolymer *in vitro* is dependent upon the number of cells placed on the biopolymer. Fewer than 200,000 cells per $5mm^2$ are unproductive. The optimal number of CHO cells seems to be about 5 times that number. MIS is detected in the media by three days *in vitro* and by implanting the polymer in animals by 7 days of *in vitro* incubation results in detectable serum MIS in the animals a week after the surgery. The production of MIS in the animals from the polymer graft is dependent upon the size of the implant and the accumulation of MIS in serum seems to be a linear function over time (Figure 1).

Most recently The team has transfected 3T3, IMR90 and BJ fibroblast cells (one mouse and two human lines, respectively) with human MIS cDNA using GenePorter, electroporation, or Fugene. Transfection efficiencies varied from very low (1-2%) for the IMR90 cells to better than 30% for the 3T3's. Attempts at electroporation with the BJ cells were unsuccessful. MIS expression was proven by MIS ELISA in 3T3 cells and 24 subclones were selected and they were propagated. MIS production per cell per day was measured for each clone and the highest producers (0.2 - 0.4 pg/cell/day) kept for further study. One such line was implanted onto the biodegradable mesh where it grew without difficulty. The mesh impregnated mesh was implanted into immune compromised mice and the mice underwent serial blood sampling until MIS was detected. Low levels of MIS (about 5 - 10 ng/ml) of serum were measured only transiently. After several weeks, the serum MIS dropped to undetectable levels despite the proliferation of 3T3 cells *in vivo*.

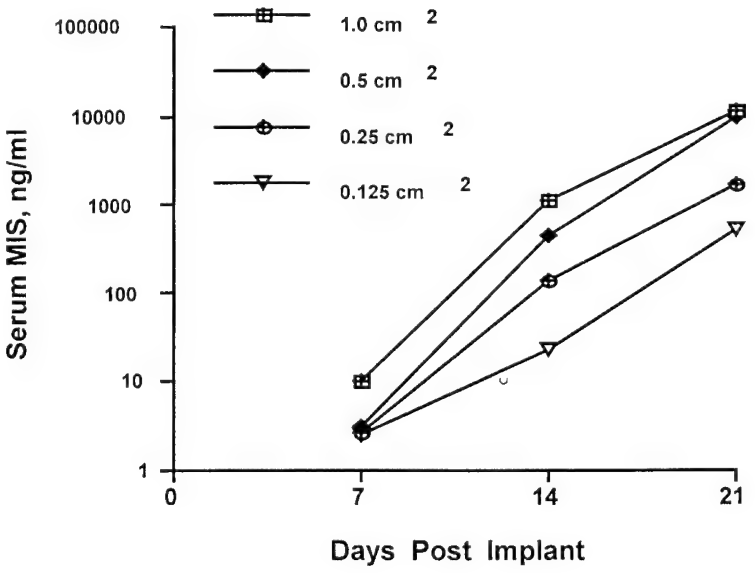


Figure 1: Serum MIS in mice implanted with MIS gene transfected CHO cells over time as a function of the size of the original implant.

Physiological levels of MIS are attained by two weeks *in vivo* only in the two larger polymer graft sizes. In addition, the MIS produced by the polymer system *in vivo* is active in the standard MIS bioassay that is highly specific for MIS.

Plan: The team has abandoned further work with the IMR90 cells as the team was not successful in achieving reasonable transfection efficiencies with the MIS gene and the team has not observed MIS secretion from these cells. Rather, the team is continuing work on the BJ and 3T3 cells, as well as harvesting mouse mesenchymal stem cells from bone marrow in collaboration with Dr. Horowitz of St. Jude's Hospital in Tennessee. These cells are much more susceptible to transfection and may be the best suited to address the issue of transfecting fibroblasts for our patients. The team will use the MIS ELISA to measure MIS production rates from non-transformed but MIS gene transfected cells. When a sufficient number of cells are amassed, the team will continue the *in vivo* studies.

Specific Aim 2: Determine if the recombinant human MIS produced by transfected CHO B9 cells or fibroblast cell lines in biopolymer matrices inhibit growth of human ovarian carcinoma cell lines transplanted beneath the renal capsule of SCID mice.

IGROV-1 Human Cancer Cells are Inhibited By MIS in vivo

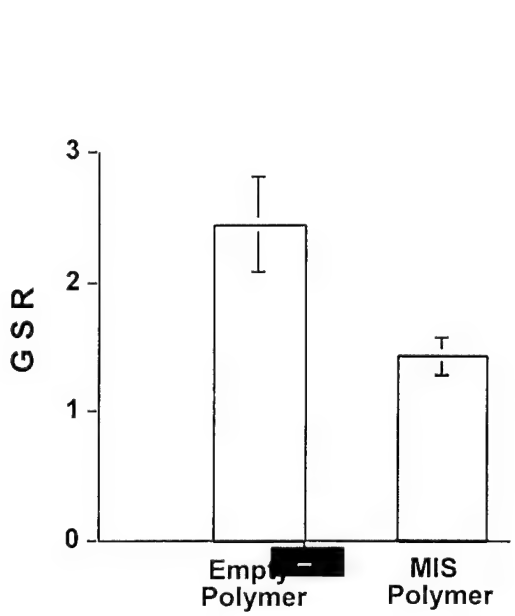


Figure 2: Graft size ratio of the ovarian cancer cell tumor IGROV-1 after exposure to MIS as compared to empty polymer

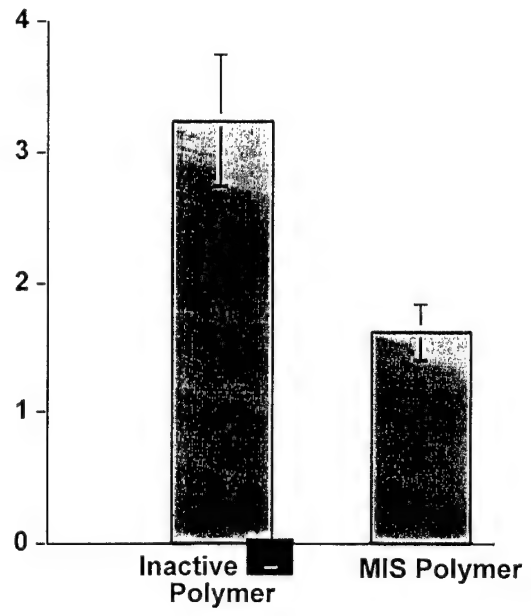


Figure 3: Graft size ratio of the ovarian cancer cell tumor IGROV-1 after exposure to MIS as compared to polymer containing cells producing inactive MIS

Progress: Human MIS delivered *in vivo via* an implanted biopolymer covered with MIS secreting cells inhibits the growth of a human cancer cell line placed as a xenograft under the subrenal capsule of the host animal (Figures 2 and 3) therefore, this phase of the studies has been completed. The success of these experiments provides the basis for one of the next series of studies in which the MIS gene will be transfected into a non-transformed cell (see below, Specific Aim II). Preferably a human fibroblast will be used to avoid immune rejection when clinical trials begin.

Plan: Once fibroblasts are successfully transfected with the MIS gene and they are proven to secrete bioactive MIS *in vitro*, the *in vivo* pharmacology and biological activity testing *in vitro* and *in vivo* will be accomplished using the same methodology that was successful for he CHO transfected cells.

Specific Aim 3: Harvest autologous fibroblasts from patients with ovarian cancer, transfect them with the human MIS cDNA, and re-implant mesh impregnated with the patients own MIS producing transfected cells into the peritoneal cavity, using laparoscopic minimally invasive techniques.

Progress: The project currently uses, for the polymer-cell graft, a partially transformed Chinese hamster ovary epithelial cell line that can be tumorigenic in immunosuppressed mice. In order to use this technology in clinical practice, the polymer-cell graft will need to consist of non-tumorigenic cells. Transfected fibroblasts grow robustly on the polymer (Detmar et al, unpublished). Once the MIS gene transfected fibroblasts are shown to secrete bioactive MIS these studies will begin.

Plan: The retroviral vector will then be used for transfection of the fibroblasts and the transfected fibroblasts tested for MIS production *in vitro*. Following documentation of MIS production by the fibroblasts, they will be grown on the polymer and the polymer-cell graft implanted into animals. The team envisions a system where a sample of the patients' own cells are transfected with the human MIS gene sequence. The cells could be fibroblasts or myofibroblasts obtained from a small skin or muscle biopsy or stem cells from peripheral blood or bone marrow. The team is now beginning to harvest mouse stem cells from bone marrow and the team will transfect them with the MIS gene and the team will then conduct the control experiments described above.

Task 8: Determine the Role of Mesenchymal Stem Cells in Fetal Tissue Engineering
Principal Investigator: N. Scott Adzick, MD, University of Pennsylvania (UPenn) and Children's Hospital of Philadelphia (CHOP) and Alan W. Flake, MD, CHOP

The overall goal of this research is to define the tissue engineering potential of mesenchymal stem cells (MSC). During this past year, the team has made considerable progress in isolating, expanding, and documenting the *in vitro* multipotential differentiative capacity of fetal liver derived mesenchymal stem cells in sheep. The team has also established site specific differentiation of adult bone marrow derived human mesenchymal stem cells in the fetal sheep model after prenatal systemic administration.

The team has successfully tissue engineered bone marrow using principles derived from known mesenchymal stem cell biology and tissue engineering. These accomplishments have been documented in a number of oral and poster presentations at national meetings as well as in manuscripts, either published or in progress.

The team has formed collaborations with Dr. Catherine Verfaillie at the University of Minnesota (human and mouse MSCs), Dr. Johnny Huard at the University of Pittsburgh (muscle progenitor

cells), and Dr. Paul Simmons from Melbourne (Mouse MSC) to investigate the relative merits of these various promising cells in our fetal models. In addition, the team is applying their low density culture techniques to sheep fetal liver derived MSC to isolate a small phenotype fetal cell with multipotential capacity. Simultaneously, the team has been developing lentiviral vectors to apply to these cells to manipulate their *in vivo* biology. The team has developed lentiviral vectors for MyoD (drives MSCs toward muscle differentiation), Pax7 (an upstream transcription factor from MyoD which regulates MSC to muscle progenitor differentiation), and are working on a vector for Hox4B (a stem cell proliferation regulatory homeobox transcription factor) which the team will test *in vivo* in future experiments.

Key Results: During the past year, the team made significant progress toward the clinical utilization of mesenchymal stem cells. Due to progress from other investigators in the field, it is clear that a mesenchymal stem cell of small phenotype, rather than the large fibroblastic phenotype used in our previous studies, has significantly greater differentiative capacity *in vitro*, and contains a higher frequency of CFU-f forming cells.

Specific Aim 1: Determine the multipotential differentiation of sheep mesenchymal stem cells *in vitro.*

Progress: The team is in the process of developing new isolation techniques in our laboratory based on those that have successfully isolated small phenotype human and mouse MSC, for isolation of similar sheep cells.

Plan: Project completed.

Specific Aim 2: Determine site specific differentiation of mesenchymal stem cells in the developing fetus.

Progress: As described above the team has now developed a number of vector systems for transducing MSCs, including AAV, lentiviral, and retroviral vectors. Utilizing marker genes the team can achieve transduction rates of 40 to 100% in large phenotype cells with these vectors. The use of these vectors with marker gene constructs will allow tracking of allogeneic MSC after either systemic or site specific transplantation.

Plan: Project completed.

Specific Aim 3: Establish clinically applicable methods to isolate and expand mesenchymal stem cells in the sheep.

Progress: As described above the team is instituting new isolation and culture techniques in our laboratory that the team hopes will ultimately provide a "state of the art" MSC for *in vivo* assessment. In human and mouse systems these cells have been documented to maintain phenotype and broad multipotentiality including endothelial, vascular, and neural, for over 100 cell doublings with tremendous expansion.

Plan: Project completed.

Specific Aim 4: Apply the principles of mesenchymal stem cell differentiation to the concept of tissue engineering by organizing mesenchymal stem cells on a biodegradable polymer and implanting the construct *in vivo* for tissue reconstruction.

Progress: The team has now developed chimeras by *in utero* transplantation and have engineered donor strain BM in their mesentery as described in previous progress reports. These animals are now undergoing transplantation with donor BM cells to assess whether there is preferential engraftment of donor marrow in the engineered BM and whether it enhances mixed chimerism in the model.

Plan: Project completed.

2.5 SIMULATION

The CIMIT Simulation Program has parallel primary thrusts: (1) developing the basic science required for realistic computer-based medical simulation and (2) validating state of the art simulations through the construction and testing of demonstration systems. The Program's principal activities involve measurement of tissue characteristics, integration of haptics into simulation, and realistic representation of medical procedures for training, device prototyping and procedural development.

Task 1: Discover Enabling Technologies for Medical Simulation
Principal Investigator: Steven L. Dawson, MD, MGH

Over the past twelve months, The Simulation Group has actively pursued both applied demonstrations of technical feasibility and cutting edge research. The team successfully fulfilled our commitment to demonstrate VIRGIL, a chest trauma training system, at the 2001 ATACCC meeting in Fort Walton Beach, Florida on September 10th, 2001. The team began active collaboration on the development of a Common Anatomic Modeling Language with Professor Daniel Jackson at the Laboratory of Computer Science at Massachusetts Institute of Technology. Also, The team began a novel research program for tissue property measurements with Professor Robert Howe at Harvard University. The team convened and chaired a two day Integrated Product Team meeting on Medical Simulation in Baltimore, bringing together the leading American simulation researchers for two days of intensive discussions which defined critical research foci. The team collaborated with members of the Regents of the American College of Surgeons to convene a two day workshop on validation of simulation for surgical training, although the meeting was postponed until Spring 2002 due to the September 11th terrorist attacks. Members of our team presented papers at numerous meetings including MMVR 2001 (Newport Beach, CA), CARS 2001 (Berlin, Germany), ATACCC 2001 (Ft. Walton Beach, FL), ITEC 2001 (Lille, France), the Stanford Workshop on Surgical Simulation (Palo Alto, CA) and at the National Library of Medicine (Bethesda, MD).

In January, Mark Ottensmeyer successfully defended his doctoral dissertation at the Massachusetts Institute of Technology, in which he presented original work he had done under the sponsorship of the CIMIT simulation team. During the year, the team also supervised two graduate students, Michal Berris from MIT, and Yann Chaumet. Through his work with us, Yann successfully completed his Master's thesis in Computer Science at the EUDIL (Ecole Universitaire d'Engenieurs de Lille).

In February, the team added two full-time senior level investigators, Mark Ottensmeyer, Ph.D. in Mechanical Engineering from MIT, and Paul Neumann, Ph.D. in Computer Science from the University of Illinois at Chicago.

Finally, in September, The Simulation Group moved to the new Partners Research Building at 65 Landsdowne Street, in Cambridge, MA. The new facilities provide state-of-the-art research areas and connectivity, with more space than was previously available at our offices in Boston.

Key Results: On September 25th, 2000, at the ATACCC 2000 meeting, the team offered to produce a prototype simulator which would demonstrate how the individual design elements of our larger research program could be unified into a working system. Our deliverable was a chest tube simulator at the 2001 ATACCC meeting, because effective training in insertion of a chest tube was a stated RAD II/Combat Casualty Care need. On September 10th, 2001, our group demonstrated the trainer to General Parker, General Kevin Kiley, Colonel James Kirkpatrick and Colonel Robert Vandre, among others, during the ATACCC meeting. The coincidence of our successful demonstration of a combat care training system which was designed to Special Forces specifications on the night before the tragic events of September 11th continues to haunt the members of the simulation team.

During the past year, the team filed seven provisional patent applications from our work. Members of our group published "Design Principles For The Use Of Simulation As An Aid In Interventional Cardiology Training" in *Minimally Invasive Therapy and Applied Technologies*, and "Designing A Computer-Based Simulator For Interventional Cardiology Training", in *Catheterization and Cardiovascular Interventions*. An editorial accompanying the latter paper called our work "an astonishing breakthrough of potentially revolutionary importance".

Specific Aim 1: Tissue Modeling - Develop tools capable of *in vivo* measurement of soft tissue characteristics, including:

- Haptics-Enabled force feedback of tissue data to render tissue manipulation realistic,
- Geometric modeling and visual feedback - recreate on the monitor screen a believable representation of tissue-tool interactions,
- Integration of physiology into computerized representations of procedures, and
- Development of a common anatomic modeling language (CAML) to achieve integration of physiology into computerized representations.

Progress:

TeMPeST

During the past year, our tissue modeling research pace has accelerated due to a combination of factors. In December, 2000, the team was awarded a four-year, $1.96 million DHRP research grant to characterize tissue properties and create mathematical models that would allow incorporation of actual tissue data into medical simulations. These funds will supplement the efforts already in place under the TATRC/RADII sponsored research to characterize *in vivo* tissue properties in animal and human subjects.

In February, the team hired Mark Ottensmeyer, Ph.D. at the conclusion of his doctoral studies in Mechanical Engineering at the Massachusetts Institute of Technology. Mark's thesis was based upon work he had done with our group, during which he created a new tissue measurement and property sampling tool, TeMPeST.

In March, the team began formal collaborations with Professor Robert Howe, Gordon McKay Professor of Engineering at Harvard University, to design and conduct a series of experiments that will allow us to characterize, over the next two years, the tissue characteristics of liver, spleen and kidney in an *in vivo* animal model. From these data, Stephane Cotin and a mathematician to be hired will derive new mathematical methods that will allow representation of the tissue properties in a real-time environment. Implementing tissue properties into medical simulations will require that the data about organ position, texture, and visco-elastic behavior be represented at a rapidly updated frame rate (~30 hertz) so that visual display will correspond to expected medical behaviors. Unfortunately, the current tools for organ deformation and display use Finite Element Methods which are not suitable for real-time interactivity rates over the complexity of surfaces which are required in medical simulation. Solving this problem will require a new mathematical method, and that effort remains a part of our overall long-term research agenda, under both the TATRC/RADII and the DHRP programs.

Our animal research this year has been performed under a research protocol that has been approved by both the MGH Subcommittee for Research Animal Care and the Defense Department's animal studies review board. Initial verification of the function and performance of the TeMPeST instrument was performed with the cooperation of the Animal Resource Center (ARC) at Dartmouth Medical School (see Figure 1). The TeMPeST was designed for use in either minimally invasive or open surgical environments, and its use was demonstrated in both capacities at the ARC. A measurement protocol was developed, and has been begun at the Harvard Center for Minimally Invasive Surgery. To date, a series of tests were performed during open surgery on porcine liver. The data (see Figure 2) show that the liver has non-linear responses to variation in both the frequency of stimulation (the TeMPeST creates a small surface vibration on the tissue) and the average preload stress applied.

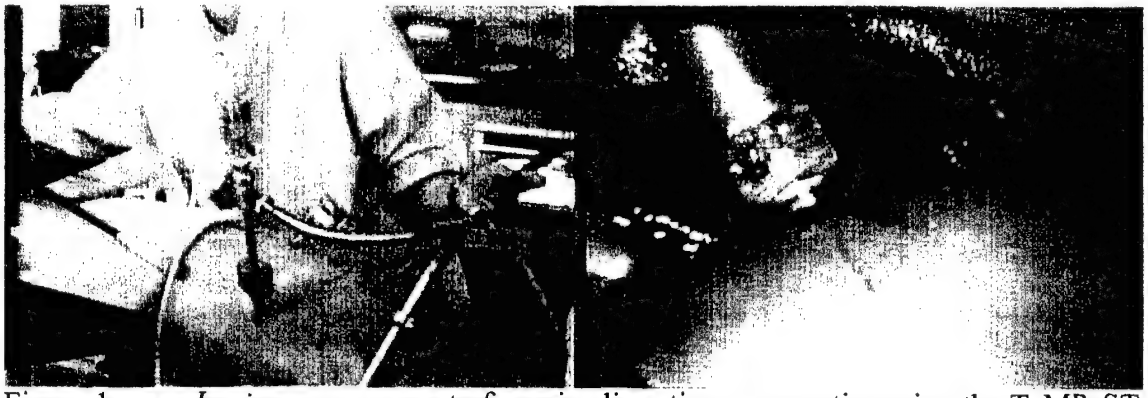


Figure 1: *In vivo* measurement of porcine liver tissue properties using the TeMPeST device. Minimally invasive TeMPeST instrument in use (left) and laparoscopic view of contact with porcine liver. Images from proof of concept testing performed at Dartmouth Medical School, Dartmouth College, Hanover, NH.

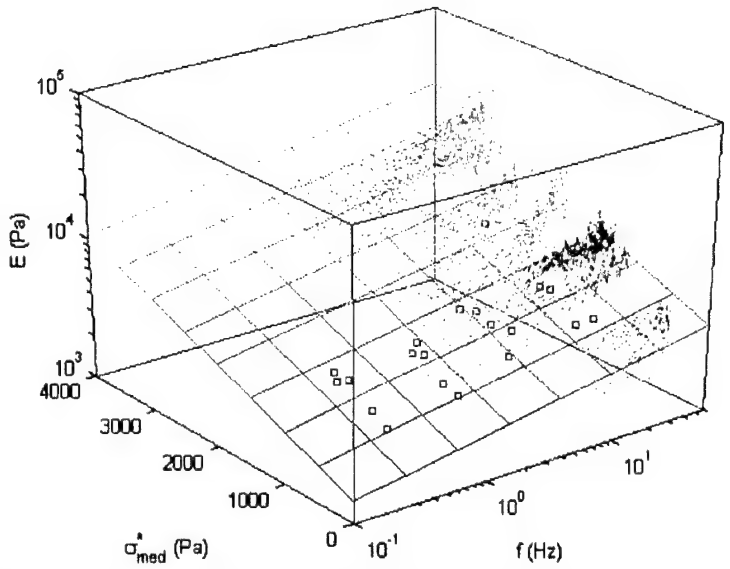


Figure 2: Elastic (Young's) modulus of living porcine liver as a function of stimulation frequency (f) and average applied stress (σ^*_{med}). Linear fit to logarithms of frequency and modulus show non-linear response of the liver tissue. Point cloud data acquired from chirp (time varying frequency) stimulation, boxes from fixed frequency sinusoidal response.

CAML

The Common Anatomic Modeling Language project entered initial research phases this year as the team began collaborations with Professor Daniel Jackson at the Lab of Computer Science at MIT. The team also hired Dwight Meglan as temporary project consultant. Together with Paul Neumann, Ph.D., of The Simulation Group, Drs. Jackson and Meglan began a series of meetings this summer to define the framework for an open architecture for medical simulations.

In June, our team participated in the Stanford Workshop on Surgical Simulation and presented our concepts for CAML. The audience consisted of academic researchers from the US and selected Asian and European countries. The audience's response reflected the breadth of the challenge that faces development of such a system, and also offered some critical comments to guide our effort. The team looks forward to bringing our future work to this group for their insight and suggestion.

In July, Drs. Neumann and Cotin participated in the National Library of Medicine's two day conference on the Digital Human at the NLM campus. The team will maintain close contact with this group, including participating with them as they develop standards for representing the Digital Human. While our goal is specifically related to creating a standard for medical simulation, the NLM group is more broadly convened, and addresses issues related to a classification system of anatomy, from the cellular to the organ and organism level. The team looks forward to collaborations with this group in areas that are of mutual interest.

Plan:

TeMPeST

During November, 2001, the TeMPeST instrument will be refurbished and reconditioned in preparation for further *in vivo* testing of porcine organs. Given the poor signal to noise characteristics of the chirp (time varying frequency) measurements, the protocol will be altered to make only selected fixed frequency sinusoidal response measurements. These tests will begin in December, 2001, and will include further tests on liver and the initial tests on spleen and kidney. The data obtained from these tests will be reduced to a functional form which can be implemented in the mathematical methods mentioned above. Where possible, investigation into the properties of diseased tissue will be pursued in an effort to expand the collection of available property data. Any animal testing not currently covered under existing protocols will begin only after approval has been obtained from all of the relevant agencies.

In addition, an instrument fabrication facility in the new research facilities at 65 Landsdowne Street will be equipped to support maintenance of the TeMPeST 1-D and the construction of additional instruments, possibly including in the short term a large (>10mm) deformation instrument proposed in the Ottensmeyer doctoral thesis or preliminary vascular property measurement instruments.

CAML

In October 2001, the team will convene a multi-institutional, international meeting in Utrecht, the Netherlands, during the MICCAI 2001 meeting. This invitation-only meeting will bring together the leading academic researchers from Harvard, Stanford and five major European centers (Zurich, Strasbourg, Nice, Tuebingen and Karlsruhe) to define the work statement for an international effort to create this fundamentally empowering tool of an open source, freely available common simulation operating system. From this meeting, the major participants (MGH, Stanford, and ETH-Zurich) will prepare a white paper that will become our seminal charter for the CAML concept.

In November 2001, the team will add Paul Sherman as a new full time member to The Simulation Group. Paul is a world recognized programming specialist who has worked with Dr. Dawson on two simulators in the past, including the acclaimed interventional cardiology system developed with Mitsubishi. Paul will assist our team in refining the software in the VIRGIL system, and in creating validation programs for the beta version of the training system. He will also assist in creating source code and a reference implementation for CAML. The CAML team will continue to work with Professor Jackson at MIT after his current academic responsibilities end following the Fall semester at MIT.

Specific Aim 2: To develop a chest tube simulation system.

Progress: The VIRGIL chest trauma training system incorporates haptics, tissue-tool interactions, real-time graphics and augmented reality to present a realistic experience of assessing and treating penetrating trauma in a simulated battlefield scenario. A free-standing but integral web-based and CD-compatible educational curriculum accompanies the training system, presenting treatment doctrine based upon the standards expressed in Tactical Medicine in Naval Special Warfare, Tactical Management of Urban Warfare Casualties in Special Operations, the

DMRTI C4 Handbook, AMEDD handbooks, and the 1988 NATO Manual. The entire system is portable and can be run on standard 110 volt AC power or as a free-standing unit for field training using an integral 12 volt DC power source (see Figures 3 - 6).

Seven provisional patent applications have been submitted, based upon the inventions created during our work on VIRGIL.

At the ATACCC meeting, General John S. Parker, Commanding General, USAMRMC, said that the VIRGIL system was so realistic that he would allow medics to treat humans unsupervised if they could pass the exam portion of the simulation curriculum. Given the General's emphatic statements concerning VIRGIL, and as a direct result of the September 11th attacks and our desire to aid America's war effort, the team has offered to temporarily redirect our efforts from our fundamental research to the delivery of a number of appropriately modified VIRGIL chest tube trauma simulators to the Army for SOFMED, 91-W and 91-B training needs. The VIRGIL system is designed to the specific battlefield treatment doctrine used by the 3/75th Ranger Battalion, with the assistance of SFC Robert Miller, and thus it may prove to be a timely method to rapidly teach special forces medics pre-deployment. At the time of this report, is awaiting the Army's response.

Whether or not the Army requests field deployable units for training, the VIRGIL system is ready for the addition of validation and metrics programs. In the coming year, if our principal focus returns to our research program, the team will collaborate with the National Board of Educational Testing and Public Policy at Boston College to create metrics methods that will allow serial exposure to the system to be recorded and errors to be classified for each individual trainee. This is an immediate outgrowth of our commitment that no simulator will be accepted by medicine until it can be validated as an effective learning system. It also reflects our core design belief the most potent application of simulation is to permit mistakes to be made. Soldiers should be able to make and correct errors safely within the simulated environment, rather than making them for the first time on a casualty in the field.

Figure 3. The VIRGIL system ready for demonstration at the ATACCC meeting on September 9th, 2001.

The mannequin consists of an anatomically accurate torso with replaceable portals in each hemithorax. Each portal is comprised of the five layers of the chest wall and provides realistic force feedback as the trainee inserts a 36 FR chest tube into the mannequin's chest cavity. The touch screen monitor integrated into the head of the NATO litter presents the trainee with the clinical trauma scenarios and leads the trainee through a series of assessment and treatment steps, from first response to tube insertion, suturing the tube in place and decisions to evacuate or observe.

Separate portals in the anterior second interspace bilaterally provide training in relief of tension pneumothorax, including visual and auditory feedback.

Correct placement of a chest tube or dart is shown through an augmented reality display on the monitor screen, which is activated as the instrument is inserted. Unlike other simulators, the VIRGIL system tracks errors in technique as they occur and will display incorrect position of the tube, including fatal errors of liver, spleen, lung or heart puncture. Trainees cannot progress to higher levels of the graded educational curriculum until they have successfully completed the lower training levels.

All control systems, including the pump systems for blood return during hemothorax treatment and the integral battery power supply for field training, are contained in the small trunk on the floor at the head of the litter.

Figure 4. The CIMIT VIRGIL development team: left to right-David Dring, Limbs and Things Ltd., Bristol, England, Mark Ottensmeyer, Yann Chaumet, Stephane Cotin, The Simulation Group, Matt Stein, Limbs and Things Ltd., Steve Dawson and Paul Neumann, The Simulation Group. In the foreground is the freestanding laptop that presents the educational curriculum which is burned onto the CD in the purple case.

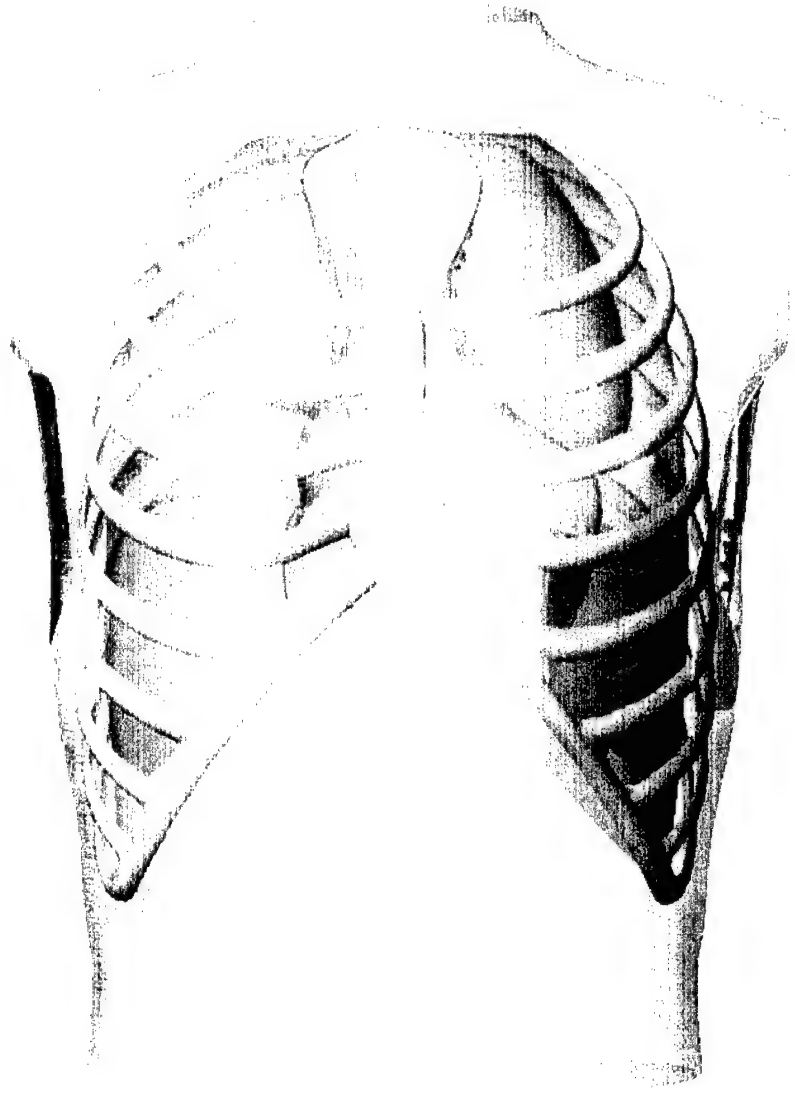


Figure 5. CAD model of a human torso used to create the mannequin torso in VIRGIL.. The anatomy represented in this torso is derived from a sequence of CT scans of a Massachusetts General Hospital physician volunteer for this project.

The anatomic surfaces represented in the CT images (skin, ribs, lungs, mediastinal and abdominal organs) were individually identified using a combination of manual and automatic segmentation techniques. These data were transferred to Limbs and Things, (Bristol, England) where the R&D team collaborated with our group to produce the CAD model shown here. The mannequin was then recreated as an exact replica of an actual male body with proportions of 73 inches height and 185 pounds weight, thus closely approximating the size and configuration of the external and internal chest dimensions of a soldier.

Note that the CAD model shows bilateral pneumothoraces. This consideration in the design permits one side of the chest to present the trainee with an easier chest tube insertion, and one side to provide a more demanding and less forgiving tube insertion. Using the control systems designed for the training system, either side can represent a hemothorax or a pneumothorax. The choice between these two scenarios is generated randomly by the software that controls the system.

Figure 6. MG John S. Parker, Commanding General, USAMRMC, after successfully placing a chest tube into the VIRGIL simulator. General Parker's favorable comments on the realism of the system were warmly received by the team members, including David Dring (rear) and Steve Dawson, team leader of The CIMIT Procedural Simulation Group.

Plan: The ACS validation meeting in Boston was scheduled for September 15 and 16th, but was postponed in response to the national tragedy of September 11th. The meeting will be held in Spring, 2002 at Boston College.

Specific Aim 3: To develop a trauma surgery decision system (TSDS).

Progress: To date the development and implementation of the TSDS has not kept pace with the tempo of the VIRGIL chest trauma training system.

Plan: Further work on this sub-project awaits additional collaboration between the CIMIT team and TATRC.

Additional plans for the next quarter include:

- To present our Year 3 continuing research proposal to TATRC and RAD II/Combat Casualty Care. This proposal will contain our 5-year research plan and also establishes our intention to form a national consortium composed of CIMIT, Stanford, Yale, MIT, and USUHS, using the expertise at each site to form a larger research effort in medical simulation technologies, and

- To continue tool design and refinement and animal experiments for tissue property measurements. An engineer and medical school graduate from Tuebingen, Germany will be joining us for one year in September to assist in tissue property measurements.

Other Work

On November 1st, 2000, Dr. Dawson presented the MSTI program and our specific plans for making medical simulation useable to the larger medical community to Dr. Thomas Russell, President of the Board of Regents of the American College of Surgeons, and Dr. Gerald Healy, Regent of the College, at a private meeting in Boston. As a result of that meeting, the American College of Surgeons has given the National Board of Educational Testing and Public Policy, our educational partner at Boston College, a grant of $45,000 to convene, with our assistance and participation, a two day summit of educators, physicians, manufacturers, and policy advisors in Boston, to define the requirements for effective validation of surgical simulation.

On January 31st and February 1st, 2001, in conjunction with TATRC, the team convened an Integrated Product Team meeting at the Baltimore-Washington International Airport Sheraton Hotel. For two days, a small group of cutting edge American researchers shared and debated insights into the major hurdles facing the advancement of medical simulation. Direct comparisons of research approaches between major institutions such as Stanford University and Harvard highlighted the spirited discussions. At the close of the second day, all participants were required to list the major unmet needs for simulation research and to identify the likely "course plot" for the coming five years in simulation research. The group's active consensus was that the major obstacles to adoption of simulation have been identified in the research protocol outlined in the MSTI program: indeed, no new research area was identified that was outside the previously enumerated program. The consensus five-year plan developed by the group found immediate practical use as the design document for the VIRGIL chest trauma training system, described above.

2.6 NEW INITIATIVES

The New Initiatives Enabling Technology provides an incubator for approaches to clinical problems that are likely to evolve into novel therapies and outcomes analyses that can be applied to a variety of medical problems. As these approaches mature, they will be made available to other Enabling Technologies.

Task 1: Lung Volume Reduction Using a Bronchoscopic Approach

Principal Investigator: Edward P. Ingenito, MD, BWH

The objective of lung volume reduction is to eliminate dysfunctional, overinflated regions of the lung. Results similar to surgical resection have been obtained by plication and stapling without tissue removal, as well as by laser directed tissue ablation. These observations suggest that removal of the dysfunctional tissue is not required. A procedure that eliminated the participation of dysfunctional tissue in the breathing process would suffice.

There have been no detailed studies on the lung mechanical effects of experimental emphysema in large animals. It is useful to know the specific changes in lung mechanics with emphysema induction to deploy the animal model for studies of novel emphysema interventions. Specifically, the team wished to understand the effects of papain to induce emphysema on airway and tissue resistance and elastance, since diseases such as emphysema may harbor both parenchymal and airway abnormalities concomitantly. To do so, the team employed a method of optimal waveform ventilation for measurement of dynamic airway and tissue mechanics, and static measurements of elastic recoil and functional residual capacity. The goal was to demonstrate that parenchymal disease induced by papain was similar to human emphysema. Hence, this project entailed two scientifically novel features: 1) the development of a reproducible model of diffuse emphysema in sheep using aerosols of papain, and 2) the characterization of the disease process using optimal waveform ventilation (OWV) in addition to static mechanics.

Specific Aim 1: To compare short term (1 month) and long term (3 month) survival, physiological responses, surgical complications and lung histopathology in control sheep (untreated, non-emphysema) following either standard surgical plication lung volume reduction or bronchoscopic lung volume reduction (BVR).

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 2: To compare short and long term survival, physiological responses, complications, and lung histopathology in sheep with emphysema (generated by papain exposure) treated either with SPVR or BVR.

Volume reduction therapy (VRT) for emphysema involves removal of hyper-expanded, dysfunctional lung, which increases recoil, improves tethering, and recruits previously compressed lung. This has traditionally been accomplished by surgical means. The team describes a bronchoscopic method of VRT in which fibrin glue containing pro-fibrotic growth factors is used to collapse and scar emphysematous lung.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Task 2: Outcome Assessment in Menorrhagia
Principal Investigator: Johanna Bosch, PhD, MGH

Minimally invasive treatments have been developed to treat menorrhagia. Evaluation studies comparing these therapies with traditionally performed therapies, such as hysterectomy, are needed to help physicians, patients and policy makers to make informed decisions. Therefore, appropriate (i.e., preference based and from the community at large) quality-of-life data and the assessed relative benefits and cost-effectiveness of new therapies are needed. In this study, the quality of life is assessed in women with menorrhagia using existing utility assessment instruments, a simple preference-based instrument (the binary-gamble method) will be developed further to assess community preferences, and a decision model to perform cost-effectiveness analyses of minimally invasive therapies for menorrhagia is being developed.

The team has developed a self-administered questionnaire including the Health Utilities Index and EuroQol-5D to collect quality-of-life data in patients with menorrhagia. The final version of the questionnaire has recently been approved by the IRB of our hospital. Recently the research staff started to recruit patients for the study. Upon data analyses of the patient survey the binary-gamble method will be used to assess community preferences for menorrhagia in a sample of the general population. A preliminary decision model has been developed in which effectiveness and cost-effectiveness of new technologies compared with hysterectomy will be compared. Model development and data collecting to feed the model, such as costs and probability estimates of major events, is currently ongoing. On completion, the study will provide quality-of-life data from women with menorrhagia and a preliminary model for cost-effectiveness analyses of minimally invasive therapies aimed at treating this condition. More generally, the study will provide information on the feasibility of using the HUI and EuroQol-5D instruments in episodic health states, such as menorrhagia, and provide a simple survey method with which to assess community preferences for health conditions analyzed in future cost-effectiveness analyses of minimally invasive therapies.

New Specific Aim: Specific Aim 3 has been added to our protocol. This Specific Aim is to develop a cost-effectiveness model to evaluate minimally invasive treatments of menorrhagia.

The full proposal includes 3 parts corresponding the specific aims of the proposal: part 1 includes a patient survey, part 2 consist of a survey in the general population, and part 3, consists of the development of a decision model for a cost-effectiveness analysis.

Key Results: Two different quality-of-life questionnaires have been composed. One questionnaire includes the standard version of the HUI (4 weeks) and the standard version of the EuroQol-5D ('your health today'). The second questionnaire includes the standard version of the HUI, but two slightly different versions of the EuroQol-5D.

Specific Aim 1: To assess quality of life in women with menorrhagia using the HUI and the EuroQol-5D.

Progress: Currently, the team has developed the questionnaire with the research staff at the DATA Group, and research staff in the Department of Gynecology to evaluate quality of life, general health status, and out-of-pocket and time costs in patients with menorrhagia. Innovative quality-of-life methods have been incorporated into the cross-sectional survey. Because standard quality-of-life instruments such as EuroQol, HUI, or the visual analogue scale are intended for chronic conditions, they are unsuitable for use for patients with menorrhagia, a condition that primarily affects women in monthly episodes. A modified version of these instruments is being used and the feasibility of this type of research will be assessed. The team believes that this type of methodology can be applied to other episodic health conditions as well. In August 2001, the IRB has approved the final version of the questionnaire. The research staff at the Department of Gynecology has started to recruit patients.

Two different quality-of-life questionnaires have been composed. One questionnaire includes the standard version of the HUI (4 weeks) and the standard version of the EuroQol-5D ('your health today'); the second questionnaire includes the standard version of the HUI, but two slightly different versions of the EuroQol-5D. The team has changed the 'today' health description to a 'bad day' health description and added a second 'good day' health description. Furthermore, patients are asked to indicate the average number of 'good' and 'bad' days per month and the number of days since their last 'bad' day with heavy bleeding. Patients are randomized to receive one of the two questionnaires. Furthermore, the team has included sociodemographic questions in the questionnaire that cover age, gender, race, and education (i.e., what is the highest year or grade in school you completed?), items on out-of-pocket expenses, and time costs to estimate patient costs.

To estimate out-of-pocket expenses, patients are asked about their extra expenses due to the condition (such as clothes, underwear, bedlinen, furniture, tampons, pads). To estimate time costs, patients are asked about the number of days unable to go to work and/or unable to do their normal activities due to the condition.

Plan: All patients presenting with menorrhagia to the Vincents' Gynecology Department at the Massachusetts General Hospital (MGH) to seek treatment for menorrhagia are eligible for the study. These patients will be informed about the study and asked to participate. If these patients are able and willing to give informed consent they will be included in the study. In 1999, more than 300 patients with menorrhagia visited the Vincents' Gynecology Department. The team

estimates that approximately 150 consecutive patients will be approached for the study; assuming 70% participation, the team anticipates that approximately 105 patients will complete the questionnaire.

Inclusion criteria: All consecutive patients presenting with menorrhagia visiting the Vincents' Gynecology Department at the MGH in order to seek treatment for their condition.

Exclusion criteria: Patients unable to give adequate informed consent. No further exclusion criteria exist.

Sample size: The team hypothesizes no substantial difference between the values obtained using the HUI and the EuroQol-5D (a linear relationship, slope=1, intercept=0). The team developed the sample size based on the standard error of the intercept and slope. The team derived these using the HUI data from the P.I.'s previous work with 88 patients suffering from intermittent claudication. In this study the within-patient standard deviation of the HUI was 0.11. Using the observed between-patient distribution in the study as the distribution of the independent variable in our linear regression, the team added normally distributed random errors (N(0, 0.11)) and simulated the results expected from our proposed study. These simulations indicate that to detect linearity between the HUI and EuroQol-5D scores, a sample size of 105 patients will give a standard error of the slope of 0.05 and a standard error of the intercept of 0.03.

Data Analysis
Upon data collections, descriptive statistics of the overall quality-of-life scores (i.e., means, standard deviations, medians, 95% confidence intervals) will be calculated and the most frequent response (mode) per attribute of the classification systems of both the HUI and EuroQol-5D will be reported. The results will be compared with published scores from women in the general population (age-matched). Multivariate and univariate regression analyses will be performed to investigate the relationship between the scores (linearity, slope=1, intercept=0) with time, age, and other potentially influential variables as independent variables. Two-way analysis of variance will be performed to test the difference between the standard EuroQol-5D scores and the adjusted EuroQol-5D scores and to test for an order effect in the administration of the HUI and EuroQol-5D.

Note: The team anticipates some delay in the recruitment of patients as Dr. Keith Isaacson, the gynecologist and co-investigator of the study, and his assistant are leaving Massachusetts General Hospital.

Specific Aim 2: To determine the relationship between the HUI and EuroQol-5D.

Progress: Specific aim 2, or "Part 2" of the study, will be done after the data collected in part 1 have been analyzed.

Plan: *General population*
A random representative sample of the general population will be recruited by random digit dialing. A professional survey firm will recruit the respondents and perform the interviews. The sample will be randomly divided into subgroups for the different mortality risks in the questions.

The chosen mortality risks in the questions will cover the range between 0 and 100% mortality risk. For example, the following risks can be chosen for the subgroups: 0.5%, 1%, 2%, 5%, 10%, 15%, 25%, 50%, 75%, 99%. In our previous study, 1000 respondents were included, divided into 10 subgroups. The required sample size for the analysis, however, should be determined.

Interview: The interview will consist of the binary-gamble method and demographic questions. The estimated duration of the interview is 10 minutes.

In the binary-gamble method each person will be presented with a health vignette describing menorrhagia.

The health vignette presenting a woman with menorrhagia will be based on the health description found in the patient survey. The most frequent answer (mode) per attribute in the classification system of the HUI and EQ-5D will be reported and used to describe the health vignette. Before using the vignette in the population survey, the description will be discussed with medical experts and adjusted if necessary. After hearing the vignette, subjects will be asked to make a hypothetical choice between the certainty of remaining in the described health state versus undergoing a treatment with specified probabilities of attaining full health or immediate death (i.e., a binary-gamble question). The possible answers to the question are: "Yes, I will take the risk," "No, I will not take the risk," and "I don't know."

Previously, the team included only one question with a single probability risk in the interview. Alternatively, the team may consider including a second, follow-up question. This second question should be similar to the first, but would present a risk one level higher or lower, depending on whether the subject accepted or denied the treatment. This approach may be more powerful but may also introduce anchoring adjustment bias after the first question. The optimal design of the method remains to be determined.

Data analysis

From the binary-gamble method with one-question per respondent, non-parametric analysis and logistic regression analysis can be used to calculate the societal mean and median utility of a health state with menorrhagia. In the non-parametric approach, a distribution of responses is constructed by calculating the proportion of yes-responses for each mortality risk. If the proportion of yes-responses is greater at the higher of two succeeding risks (which is illogical since the proportion choosing the treatment should decrease as the mortality risk increases), the responses are pooled for the two risk levels and the proportion is recalculated. Once the distribution curve is constructed, one obtains the empirical "survival-function" and the mean utility can be estimated by the area above the distribution curve (1 minus the area under the curve). The median utility corresponds to 1 minus the risk level (i.e., midpoint of a risk-interval) at which 50% would accept the bid according to the distribution curve. Multivariate logistic regression analysis is performed to assess the mean utilities parametrically. To estimate the parametric mean utility, the study sample means of the covariates can be included in the resulting regression models and the dependent variable will be plotted as a function of risk . The area above the curve represents the mean utility.

The calculated scores will be compared with the HUI and EuroQol-5D scores assessed in the patient survey. Furthermore, for validation purposes, the team will add a second health description which was used in the previous study (i.e., amputation due to peripheral arterial occlusive disease). The results of the current study will be compared with the results from the previous study.

The binary-gamble method with two-gamble questions per respondent, will yield censored data in a single-bounded interval and a double bounded interval, which allows other methods for data-analysis (e.g. based on Kristrom Spike Model, or Weibull model).

Specific Aim 3: Develop a cost-effectiveness model to evaluate minimally invasive treatments of menorrhagia.

Progress: A preliminary cost-effectiveness model of uterine artery embolization (UAE) compared with hysterectomy for patients with fibroids, which is the single largest cause of menorrhagia, has been developed. Using the best estimates for cost and effectiveness the team found so far in this model, UAE had slightly lower mean cost and higher mean effectiveness than hysterectomy ($3,975 and 22.66 QALYs compared to $4,084 and 22.23 QALYs, respectively), making it a cost-saving alternative. This "base-case" analysis assumes that UAE has a low technical failure rate of only 2%, that it is effective in 90% of patients, and that fibroids recur in 0.1%. If the fibroids do not recur after successful embolization, then the mean cost decreases to $3,877 and the mean effectiveness increases to 22.80 QALYs. If embolization is only effective in 75% of patients, then it is no longer cost saving, but the incremental cost-effectiveness ratio (ICER) is $3,397/QALY.

This model, by necessity a simplification of realty, demonstrated minimal differences in costs and effectiveness between the two procedures. These results are likely to change after varying the assumptions about the costs and effectiveness of both procedures. Further development of the model, including more recent data and details about complications, procedures, and costs in the follow up, are needed.

Plan:

Model Development

A state transition (Markov) decision model will be developed in order to compare initial and long term benefits and costs of minimally invasive treatment strategies, such as cryoablation, thermoablation, laser ablation, trans-cervical endometrial resection or ablation, radiofrequency ablation, and uterine artery embolization with the traditionally performed hysterectomy in patients with hemorrhagia. The model will contain a finite number of health states describing the consequences of possible clinical events. Secondary treatment and follow-up events will be built into the model. Transitions between each health state will be specified over a particular fixed time frame (i.e., cycles) by assigning different probabilities for each form of management under evaluation. The construct of the model will be based on literature and discussion with clinical experts.

The cost-effectiveness analysis will be performed according the guidelines of the US Panel on Cost-Effectiveness in Health and Medicine. In the analysis, the team will estimate the total costs

and effectiveness (in terms of quality-adjusted life years) of the initial procedures and events during follow-up. In addition, incremental cost-effectiveness ratios (additional costs divided by the effectiveness gained in comparison to the next best strategy) will be calculated. The cost-effectiveness analysis will be performed from the societal perspective. Sensitivity analyses will be performed by varying the effectiveness and costs estimates.

Data

Effectiveness: Quality-adjusted life years will be estimated for each treatment strategy on the basis of morbidity and mortality data, other clinical outcomes, and quality-of-life outcomes. Effectiveness data will mainly be retrieved form the literature and experts. Pre-treatment quality-of-life data will be collected form patients and the general public (the proposed study).

Costs: Costs incurred by the hospital and patients will be included in the model. Hospital costs of procedures and events in follow-up will be retrieved from the hospital accounting system (Transition Systems, Inc. TSI). In sensitivity analysis, the team will use a range of costs retrieved by Medicare reimbursements for CPT-4 codes or DRG codes and the literature. Patient costs will include time costs and out-of-pocket expenses. Time costs will be estimated by multiplying the time expended for the intervention by the average wage for these women (Bureau of Labor Statistics). Out-of-pocket costs and time costs during follow-up will be estimated from the patient questionnaire and an ongoing study in the Department of Gynecology.

3.0 CLINICAL CHALLENGES

3.1 TRAUMA AND CRITICAL CARE

The Trauma and Critical Care Clinical Challenge is developing new, efficient technologies that reduce morbidity and mortality from trauma and critical illness. These developing technologies lessen: the time required for recovery, the pain and suffering associated with therapy, and the overall cost of patient care.

Task 1: Microsensors – Real-Time Blood Assay
Principal Investigator: Christopher Dube, PhD, Draper Laboratory, Cambridge, MA

The goal of the project is development of a microarray sensor technology that is capable of measuring a detailed signature profile of blood (or other body fluid) components in near real-time. Components under investigation include both soluble proteins and microbial pathogens. The project is driven by several needs: 1) ICUs need more detailed, timely information on the metabolic, inflammatory, or infectious state of a patient, 2) Near real-time serum level of indicator proteins (e.g. Parathyroid hormone (PTH) level during parathyroid surgery), and 3) Faster detection and identification of blood-borne infectious disease. In particular, the impact of development of the later is significant in that it would revolutionize diagnostic microbiology from current culture-based methods to faster, more precise, more sensitive technology. Through our collaboration with Dr. Stephen B. Calderwood, Chief of the Division of Infectious Disease at MGH, the team has identified specific clinical applications of our sensor technology. These focus largely on detection and identification of blood-borne infectious disease. If successful, a direct-read, near real-time detection and identification of human pathogens will revolutionize diagnostic microbiology from largely culture-based methods to a detection/identification approach that is highly specific in its ability to discriminate pathogens with a technology platform than can provide sensitive measurements in a short period of time.

Key Results: In the last year the team accomplished a number of noteworthy milestones, and the team is positioned to apply this year's experience to the detection of E. coli bacteria in the next several weeks. The key milestones of the past year include; Biological detection of the immunoprotein IgG using individually functionalized μCANARY element. Biological detection of an immunoprotein using individually functionalized μCANARY elements came as a result of the progress made during this same period in developing surface chemistry for antibody attachment to the sensor elements. The first biological detection of *Legionella pneumophila* antigen in urine using the 9-element μCANARY sensor. The 9-element μCANARY sensor was functionalized with four different antibodies (two polyclonal and two monoclonal) using BioDot addressing of individual sensor elements. The Legionella and IgG experiments described here represent the first time that individual elements of the μCANARY have been functionalized and demonstrated selective response. Correlation of the frequency shift of binding of biologically labeled particles with the optical density of the bound particles. This represents a major achievement in that it was an independent means of quantitation of the sensor frequency response to a biological target with an independent measure of binding (optical density) of the biological target to the sensor elements. The first reproducible means of regenerating the sensor

surface for recycling of μCANARY sensors. Finally, Draper Laboratory began funding Prof. David Kaplan at Tufts University to develop alternative affinity ligand reagents (ALRs) for the detection of microorganisms, based on peptides expressed on the outer surface coat of phage. These ALRs will be used in conjunction with the μCANARY sensors for detection of microbial pathogens.

Specific Aim 1: Determination of analytes of interest and detection requirements. To accomplish this Specific Aim the following items will be addressed:

- Characterize receptor coating application for microbial pathogens,
- Sensor system development,
- Sensor surface chemistry,
- Characterization of exposure to laboratory samples,
- Determine level of accuracy provided by technique,
- Exposure to fluid samples with unknown concentrations,
- Development of hardware, and
- Development of software.

Analytes of interest and detection requirements

Progress: During the past year the team substantiated the biological detection of *Legionella pneumophila* antigen using the 9-element μCANARY. In this experiment all of the antibodies were tested (Table 1 and Figure 1). Only the polyclonal sheep antibody bound the Legionella antigen, as evidenced by a frequency shift concurrent with exposure. This represents the first time that individually functionalized sensor elements of Draper Laboratory's 9-element μCANARY sensor demonstrated selective response to a biological target. The Legionella work was done in collaboration with our MGH partner Dr. Stephen Calderwood and his group.

A second major development within this subtask was the *visualization* of selective sensor element response using biologically modified particles of polystyrene, and quantitation of the optical density, and hence the number of bound particles. In this work, 1 μm polystyrene particles modified with rabbit anti-IgG were detected by the frequency response of the selectively functionalized μCANARY sensor. Elements 1,3,7,9 were blocked with NGS (or BSA) to inhibit particle binding and elements 2,4,6,8 were functionalized with IgG. A striking phenomenon observed with this experiment was the pattern of the particles on the IgG labeled sensor elements (Figure 2). The alignment spacing correlates with the front surface electrode pattern used to excite the piezoelectric film. This striking behavior is under further investigation.

Figure 1. Bound anti-IgG particles on IgG functionalized sensor element (E4).

Characterize receptor coating application for microbial pathogens

Progress: Coating procedures to apply ALRs to the μCANARY sensor surface to detect *Legionella pneumophila* are complete. Optimal performance of the antibodies are achieved with aminoethanethiol as the primary coating on the sensor, followed by covalent attachment of the antibody with a glutaraldehyde linker. Other surface attachment strategies (such as using protein G or MUA/PLL) gave similar performance in terms of protein attachment.

Plan: During the next quarter the team plans to:

- Continue to improve surface chemistry and bioassay technologies for better uniformity of protein binding. Includes imaging and modeling of particle attachment (polystyrene and *E. coli*) on sensor surface,
- Perform experiments using the BioDot™ and a flow cell for immobilization on 9-element sensor arrays,
- Optimize the coating procedure to apply ALRs to the μCANARY sensor surface to detect *E. coli* whole organism,
- Perform preliminary studies to implement the E. coli genomic typing platform of with the μCANARY sensor system for direct detection of DNA hybridization of the organism's genome. The project entails extraction of the E. coli DNA, amplification, and detection. Detection and identification of the bacteria will be by detection of DNA hybridization between products from genomic DNA of strains of E. coli (strain O157:H7) and DNA sequences from each of the eight polymorphic regions of this E. coli genome, fixed to the sensor chips. The team will use polymerase chain reaction (PCR) to amplify the DNA prior to sensor exposure, and
- Determine the selectivity and efficiency of the ALRs to detect the *E. coli* whole organism.

The team has identified three strains of *E. coli* through our Tufts University collaborator, Prof. David Kaplan, who is working to develop alternative affinity ligand reagents (ALRs) for the detection of microorganisms. These include F pili (ATCC 29839), another producing an O-antigen- a polysaccharide component of the LPS (ATCC 12014), and the wild type strain K12 that lacks F pili and the O antigen. The Draper/MGH/Tufts team has obtained relevant antibodies through commercial sources and these will be evaluated in the next quarter.

Develop sensor regeneration method

Progress: Insufficient progress with defining a chemical means of sensor surface regeneration led to identification of *any* means of sensor surface regeneration. Excellent results have been achieved with oxygen plasma cleaning of the surface. Results using optical microscopy, sensor frequency response, and bioassay on the plasma cleaned surface confirms the efficacy of plasma cleaning for sensor regeneration.

Plan: Project completed.

Characterization of exposure to laboratory samples

Progress: Detection of *Legionella* (repeated) has been confirmed for the sheep polyclonal antibody using BioDot addressing of the μCANARY 9-element sensor.

Plan: During the next quarter the team plans to:

- Implement a reference sensor algorithm to improve signal-to-noise by elimination of common mode effects,
- Implement detection of E. coli whole organism detection using selective antibodies bound to μCANARY 9-element sensor and determine the reproducibility and LDR of the sensor to samples containing *E. coli* whole organism,
- To determine the selectivity of the sensor in detecting *E. coli* by incorporating the different strains of E. coli to test for nonspecific binding of the *E. coli* antibodies. Note that this is a shift by the team to consolidate our efforts on one organism, E. coli, rather than *Legionella pneumophila, Cryptococcus neoformans,* and *E. coli*, and
- If time and resources permit, implement genomic typing of E. coli by detection of DNA hybridization between products from genomic DNA of strains of E. coli (strain O157:H7) and DNA sequences from each of the eight polymorphic regions of this E. coli genome, fixed to the sensor chips. The team will use polymerase chain reaction (PCR) to amplify the DNA prior to sensor exposure.

Determine level of accuracy provided by technique

Progress: The integrated optical density of the biologically-modified bound particles on the sensor elements, and hence the number of bound particles, agreed well with the frequency response of the elements, as shown in Figure 3. This provides an independent confirmation of the sensor response.

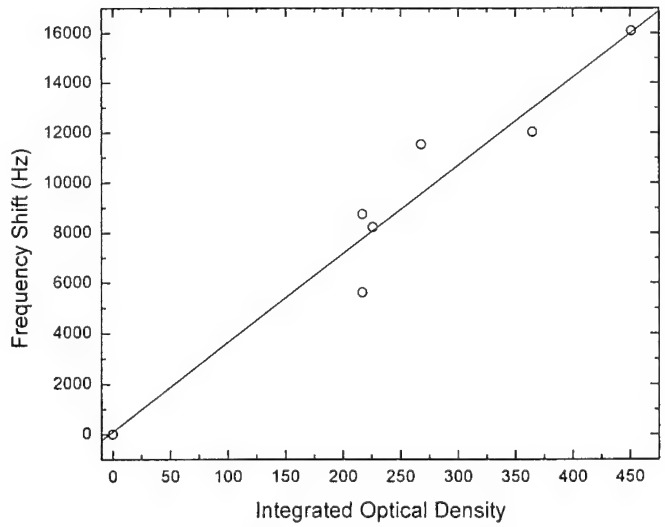


Figure 2. Correlation of sensor frequency response and integrated optical density of biologically labeled particles.

Plan: During the next quarter the team plans to:

- Use the 9-element sensor array, to obtain the reproducibility and linear dose-response curves of the sensor when exposed to *E. coli*, which enables the determination of the detection limit and the accuracy level of the sensor, and
- To compare ELISA response and μCANARY response for the *E. coli* samples.

Exposure to fluid samples with unknown concentrations
Progress: No activity this quarter

Plan: During the next quarter the team plans to obtain the linear dose-response curves of the sensor for detection of *E. coli*. Fluid samples containing the bacteria with unknown concentrations will be used to challenge the sensor.

Development of hardware
Progress: A total of 70 9-element μCANARY sensors designed for liquid exposure experiments have been fabricated around the open-loop testing electronics. The team has completed a preliminary design and started build of closed-loop electronics for testing 9-element devices. All the parts have been ordered for building the closed-loop electronics, including circuit boards and components and new packages. Further development of the closed-loop electronics will not be pursued at this time in an effort to focus activities on validation of μCANARY sensors for proteomic and genomic detection and identification of *E. coli*.

The team has evaluated all 9-element sensors for performance in both air and under liquid-exposure conditions. A number of devices exhibited poor mode structure. The problem with leakage seen in the previous quarter has been resolved by yet a further refinement of μCANARY device attachment. Poor mode structure typically means that those elements cannot be used for immunoassay. The cause of the poor mode structure is under investigation but is likely due to uniformity of the AlN film.

Plan: Under Draper Laboratory funding during the next quarter the team plans to:

- Manufacture microfluidic interfaced 9-element μCANARY sensors for reduced reagent volume and faster sensor response.

Development of software
Progress: The team is working to develop data analysis algorithms for implementation of the closed-loop testing, and is expected to give a noise reduction for a typical device from about 3 ppm to 0.1 ppm. The team has completed the closed-loop algorithm for a dual-element sensor. and the same approach will be implemented with the μCANARY.

Plan: There is no plan at this time to implement completion of software for closed-loop μCANARY sensor interrogation since focus during the coming year does not include completion of closed-loop electronics hardware.

Task 2: Application of Microwave Imaging to Rapid Non-Invasive Detection of Intracranial Hematoma
Principal Investigator: Lt Col Geoffrey S. F. Ling, MD, PhD, Uniformed Services University of the Health Sciences, (USUHS), Bethesda, Maryland

Over the past year, work on the radio frequency (RF) triage system (RAFTS) has continued. Over this past quarter, the team was successful in obtaining USA-MRMC Human Use IRB approval to initiate human clinical trial.

For initial human clinical studies, both USUHS Human Use Institutional Review Board (IRB) and by the U.S. Army-Medical Research and Materiel Command's (USA-MRMC) IRB has given full approval. The RAFTS project has received a less than minimal risk designation.

Since USA-MRMC Human Use IRB approval was received in August, 2001, the team began preparation for conducting the normal human clinical subjects study. This entailed subject recruitment and establishing the test site. A frame was made to hold the RAFTS in a common position. A bed was modified to remove any metal parts that might interfere with RAFTS signal. As these preparations are now complete, the team anticipates the study will commence shortly.

Key Results: This past year, the team reported success in completing study of the RAFTS as it is applied to diagnosis of intracranial hematomas, pneumothorax and compartment syndrome. In brief, the team reported the findings of *in vitro* testing using cadaveric pig brains. The results from this work demonstrate that the RAFTS can differentiate hematomas from brain and skull. Subsequently, the team reported the completed *in vivo* study that was performed in live anesthetized pigs. These studies show that the RAFTS can accurately detect the presence of hematomas at epidural, intraventricular, subdural and intraparenchymal sites in a clinically relevant model. Also in pigs, RAFTS can also detect as little as a 10% pneumothorax and as little as 2cc of either blood or saline in the muscle compartment

Specific Aim 1: To demonstrate the feasibility of applying the microwave diagnostic tool to accomplish the following:

- to identify pneumothorax,
- to identify the presence of blood in the epidural space,
- to identify compartment syndrome,
- to detect intraventricular hematoma, and
- to detect intraparenchymal hematoma.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 2: To perform additional *in vivo* studies of pneumothorax and compartment syndrome in pigs using the RAFTS system.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 3: Using RAFTS, perform baseline studies in normal human volunteers to determine the RF signals under normal uninjured conditions:

- Determine baseline signature of the head,
- Determine the baseline signature of the chest,
- Determine the baseline signature of the leg, and
- Characterize RAFTS signal response to blood, bone and bone-blood interface.

Progress: No progress to date regarding the human clinical trial as final IRB approval was obtained about 1 month ago.

Clinical Study

Human use institutional review board (IRB) approval for a clinical study using the RAFTS to be conducted at USUHS has been received from USUHS and USA-MRMC. The initial evaluation of the proposed study to examine the head, chest and leg of 15 normal human volunteers has been submitted. The purpose of this study will be to obtain baseline values. The hypothesis is that there are no significant differences in the RAFTS signature for head (or chest or leg) among normal humans. If this proves to be true, then clinical trial in trauma patients can be prepared. If this proves to be false, then modifications of the trauma clinical trial will be needed, such as side-to-side comparison.

Plan: The purpose of this study will be to obtain baseline values of the head, chest and leg of 15 normal human volunteers. The hypothesis is that there are no significant differences in the RAFTS signature for head (or chest or leg) among normal humans. If this proves to be true, then clinical a trial in trauma patients can be prepared. If this proves to be false, then modifications of the trauma clinical trial will be needed, such as side-to-side comparison.

Task 3: Near-Infrared Reflectance Spectroscopy (NIRS) to Assess Regional Ischemia both during Trauma Resuscitation and at the Bedside in the Intensive Care Unit
Principal Investigator: Juan Carlos Puyana, MD, BWH

The overall objective of this research effort is to use Near-Infrared Reflectance Spectroscopy NIRS and other new minimally invasive technologies to determine the severity and reversibility of hemorrhagic shock by means of assessing organ specific cellular function and metabolism.

Presently the team is focusing on establishing the effects of spontaneous breathing on tissue blood gases. Previous experiments so far have shown that the PCO_2 of the muscle correlates well with the severity of shock. However these data has not been obtained in shocked animals without ventilatory support and without controlling for ventilation and arterial CO_2

Dr. Puyana has moved to the University of Pittsburgh where he plans to continue this research.

The work completed so far has allowed us to show that the use of a multi-parameter sensor facilitates the identification of a specific organ response to hemorrhage. That changes in baseline tissue PCO_2, PO_2 and pH occur promptly after hemorrhage and that these responses are similar in all organs studied (liver, gut peripheral muscle, and stomach). Also, the team has demonstrated that changes in tissue pH and PCO_2 in peripheral muscle correlate well with severity of blood volume lost and with the resuscitative interventions used to replace hypovolemia.

Key Results: This past year, the team tested the concept that the animals breathing spontaneously may be capable of compensating the elevation of tissue PCO_2 by reaching high respiratory rates and induced compensatory hyperventilation. To test this hypothesis the team used a rat model of uncompensated shock. A multiparameter (PO_2, PCO_2, pH) monitoring fiberoptic catheter was used for the assessment of tissue perfusion during shock.

Specific Aim 1: To develop minimally invasive techniques to measure peripheral organs (Bladder) pH in the victim of hemorrhagic shock and evaluate the potential of this method as a predictor of multiple organ dysfunction syndrome (MODS) and a guide for resuscitation.

Progress: The team is awaiting Partners Risk Assessment approval for human studies.

Plan: Complete risk assessment process and continue with human studies phase of project.

Specific Aim 2: Validation of NIR tissue pH measurement

To develop new strategies aimed at protecting or improving cell/ organ tolerance to shock and hypoperfusion, the team will continue my work on the use of Pyruvate ethyl ester as a modulator of the oxidative stress response to shock induced ischemia.

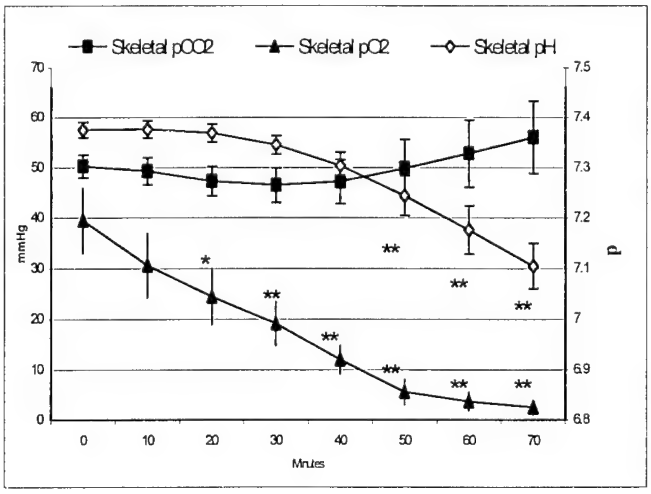
Dr. Puyana has contacted the group from TherOx and they are committed to provide me with the equipment necessary to test the use of liquid oxygen in a resuscitative strategy in an animal model of hemorrhage using TherOx technology. This work is scheduled to start in October.

Progress: Tissue monitoring may allow for the early detection of poor perfusion. The team has previously shown that tissue PCO_2 and pH are good indicators of the severity of blood loss and adequacy of resuscitation using a ventilated swine model. In the spontaneously breathing animal, however, these relationships are not as well-defined. Here the team investigated the effects of spontaneous ventilation on monitoring skeletal muscle (SM) tissue gases during hemorrhagic shock..

Methods: 12 male Sprague-Dawley rats (250-350 g) were anesthetized with pentobarbital (60 mg/kg, IP). A tracheostomy was performed and animals were allowed to spontaneously breathe room air. A femoral and carotid catheter were placed for monitoring and blood withdrawal. A Paratrend7 sensor (Diametrics Medical, MN) was placed in the adductor muscle to measure tissue gases. Animals were bled over 10 min until reaching a MAP of 40 mm Hg, and then maintained at this pressure for 1 hr by withdrawing or infusing blood as necessary. Data was analyzed using repeated measures of ANOVA with a Newman-Keuls test for significance (*$p<0.05$, **$p<0.01$). Correlations were made using linear regression.

Results: The SM pCO_2 decreased slightly during the initial 40 min of hypotension and was associated with significant tachypnea (78 ± 4 vs. 98 ± 6, $p<0.05$) and a decrease in $PaCO_2$ (34 ± 2 vs 16 ± 1, $p<0.0001$). SM pO_2 decreased rapidly and became significant within 20 min. SM pH fell slowly and only became significant after 50 min. A strong correlation between blood volume loss and ΔpH was observed at the end of shock ($r^2 = 0.74$, $p<0.01$).

Conclusions: In the spontaneously breathing animal, hemorrhagic shock is associated with early tissue hypoxia, but the previously observed hypercarbia does not develop. Shock-induced hyperventilation may ameliorate SM hypercarbia and dampen the severity of tissue acidosis seen early in shock. ΔSMpH at the end of shock, however, remains a good indicator of the severity of blood loss.



Plan: Based upon these findings, the team will continue to study the compensatory changes during shock that may impact on tissue gas analysis. The team will concentrate in the use of pH and use this information along the data presently being collected in the swine model where the team continues to study the sensitivity and specificity of the sensor incorporated in a modified bladder catheter (smart Foley).

Task 4: Noise-Enhanced Tactile Sensation for the Management of Sensory Deficits in Patients with Stroke

Principal Investigator: D. Casey Kerrigan, MD, Spaulding Rehabilitation Hospital (SRH), Boston, MA

The overall goal of this research is to gain an increased understanding of how noise affects the detection and discrimination of mechanical cutaneous stimuli in subjects with impaired sensation, and also to establish a scientific foundation for the development of a clinically useful noise-based technique for improving tactile sensation in humans.

Key Results: The reconsenting of the twenty-one subjects involved in this project, with the revised DoD protocol and consent form, continued during this quarter.

Specific Aim 1: Design and construct a suitable apparatus for patient experiments.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 2: Demonstrate the feasibility of both the apparatus and an experimental protocol through implementation in patients with stroke; collect data on patients with stroke.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001

Specific Aim 3: Analyze data to test the hypothesis that electrical noise can enhance the ability of patients with stroke to detect subthreshold mechanical stimuli.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

3.2 VULNERABLE PLAQUE PROGRAM

During the past year the Vulnerable Plaque Program of CIMIT has made significant advances.

Below is the list of projects funded for FY 02:

- Detection of Vulnerable Plaque using Optical Coherence Tomography
- CT and MRI Imaging of Vulnerable Plaque
- Detection of Vulnerable Atherosclerotic Plaques with Radionuclide Technology
- MRI of Carotid Plaque
- Treatment of Vulnerable Atherosclerotic Plaques with Macrophage-Targeted Photodynamic Therapy,
- Magnetic Resonance Imaging of Vulnerable Plaque, and
- Vulnerable Plaque Program Outcomes – Technology Assessment.

With the relocation of Central CIMIT to the Cambridge site, the Vulnerable Plaque Program has now been established in the former CIMIT space of approximately 2000 sq. ft. at Charles River Plaza. This space provides a synergistic home for cardiologists, radiologists, Ph.D. scientists, post-doctoral fellows and students working in many aspects of vulnerable plaque research. This stimulating environment is felt to be key to the future success of the Vulnerable Plaque Program.

Task 1: Vulnerable Plaque Detection and Treatment
Principal Investigator: James E. Muller, MD and Thomas J. Brady, MD, MGH

The original programmatic aims of the Vulnerable Plaque program have been accomplished. In addition, the number of faculty, PhD fellows and students in the program increased significantly during the past year. The following represents the programmatic accomplishments.

Specific Aim 1. Establish a Vulnerable Plaque Lecture Series.

Progress: During the past year the Vulnerable Plaque Program launched its weekly seminar series. The program consisted of a weekly series of topics on various aspects of vulnerable plaque given by investigators within and outside the Boston community. The lecture-discussion format enabled the transfer of ideas and generated provocative interaction. A written summary of each presentation was obtained as well as a video of the slide presentations which are available to our corporate sponsor.

Plan: To continue the VPP Lecture Series.

Specific Aim 2: Establish an International Symposium on Vulnerable Plaque.

Progress: Dr. James Muller along with Dr's Peter Libby and Valentin Fuster created the First International Symposium on Vulnerable Plaque. The symposium will be held in Cambridge, MA on October 4 – 5, 2001 at the MIT University Park Hotel. Dr. Muller was able to recruit key investigators from around the world. The program is divided into three half-day sessions which will concentrate on critical aspects to the vulnerable plaque including biology, detection and therapy (Appendix 2 - program). The speakers are all experts in their fields. The presentations

are limited to 10 minutes with ample time for discussions, which are expected to be lively and reveal controversial opinions in critical areas of research opportunity. The entire program will be available on a searchable CD by the end of the year.

Plan: To coordinate a second international VP Symposium for the Spring 2003.

Specific Aim 3: Obtain Additional Funding for the Vulnerable Plaque Program.

Progress: In addition to the DoD-CIMIT support, leadership of the Vulnerable Plaque Program have generated over $600,000 from industry to support additional activities of the vulnerable plaque program including additional pilot studies within CIMIT.

Plan: To continue to seek sponsorship and active collaboration with partners in industry whose companies have an interest in the detection and/or treatment of vulnerable plaque.

CIMIT Vulnerable Plaque Detection & Treatment Program Lecture Series
September 2000 – May 2001

September 11th	Thomas J. Brady M.D. and James E. Muller, M.D. "Overview of Vulnerable Plaque Program" MGH
September 18th	Richard Pasternak, M.D. "Do the Statins Stabilize Vulnerable Plaques?" MGH
September 25th	Gary Tearney, M.D., Ph.D. "Optical Coherence Tomography" MGH
October 2nd	Jeffrey Raines, M.D., Ph.D. "Estimating Atherosclerotic Burden and Endothelial Function by Non-Invasive Methods" University of Miami
October 16th	Richard T. Lee, M.D. "The Biochemical Basis of Plaque Stability" BWH
October 23rd	Ik-Kyung Jang, M.D., Ph.D. "First Human Application of OCT" MGH
October 30th	Campbell Rogers "Methods to Ablate Atherosclerotic Plaque"

BWH

November 6th — Sergio Waxman, M.D.
"Angioscopy for Disrupted and Vulnerable Plaque"
University of Texas

November 20th — Walter Koroshetz, M.D.
"Significance and Detection of Vulnerable Plaque in the Carotid Arteries"
MGH

November 27th — James Januzzi, M.D.
"Serum Markers of Plaque Vulnerability"
MGH

December 4th — Michael Feld, M.D.
"Raman Spectroscopy"
MIT

December 11th — Michael Johnstone, M.D.
"A Rabbit Model of Vulnerable Plaque and Triggering"
BIDMC

January 8th — Carey Rappaport, Ph.D.
"All Set to Explode-Applying Land Mine Detection Technology to the Vulnerable Plaque Program"
Northeastern University

January 22nd — Paul Yock, M.D.
"Intra-coronary Ultrasound and Other Methods to Characterize Coronary Plaques"
Stanford

January 29th — R. Rox Anderson, M.D.
"Optical Treatment for Plaque: Vulnerable Ideas from a Dermatologist"
MGH

February 5th — Thomas Aretz, M.D.
"Pathologic Features of Vulnerable Plaque"
MGH

February 12th — Charles DiMarzio, Ph.D.
"Hyperspectral Imaging - Potential for Detection of Vulnerable Plaque"
Northeastern University

February 26h — Rene Botnar, M.D.
"Coronary Wall/Plaque Visualization, Aortic Plaque

	Imaging, and Assessment of Plaque Disruption by MRI" BIDMC
March 5th	Andrew Selwyn, M.D. "Early Detection of Vasculopathy by Brachial Artery Reactivity Studies" BWH
March 12th	Peter Stone, M.D. "Coronary Artery Flow Profiling to Predict Vulnerable Plaque Formation" BWH
April 2nd	Chris O'Donnell, M.D. "Epidemiology of Subclinical Atherosclerosis: Implications for Prevention and Treatment of the Vulnerable Plaque" MGH
April 9th	Tony Rosensweig, M.D. "Gene Therapy for Vulnerable Plaque: Theoretical Considerations" MGH
April 16th	no meeting
April 23rd	Yadon Arad, M.D. "Coronary Calcification: A Marker for the Vulnerable Plaque or the Vulnerable Person" College of Physicians and Surgeons of Columbia University
April 30th	Discussion of ACC Vulnerable Plaque Abstracts
May 7th	Rob Gertszten, M.D. "Monocytes, Chemokines, and Atherogenesis: Potential Therapeutic Interventions" MGH
May 14th	Johanna Bosch, Ph.D. "Cost-effectiveness of New Technologies in Detecting and Treating Vulnerable Plaque in CAD" MGH
May 21st	Karen Furie, M.D. "OCT for Characterization of Carotid Vulnerable Plaque" MGH

CIMIT 2001 Vulnerable Plaque Conference:
Pathophysiology, Detection and Treatment
October 5 & 6, 2001
Cambridge, MA

Friday, October 5th

7:30 am - 7:50 am	Registration and Continental Breakfast
7:45 am - 8:00 am	Welcome – Dr. John Parrish, Director of The Center for the Integration of Medicine and Innovative Technology (CIMIT)
Morning Session:	***The Pathophysiology of the Vulnerable Plaque***
8:00 am - 8:10 am	Opening Remarks - Dr. James Muller, Chair of Session
8:15 am - 8:45 am	Dr. Peter Libby - *Biochemical and Cellular Basis of the Vulnerable Plaque*
8:50 am - 9:10 am	Dr. Renu Virmani - *Pathologic Features of Vulnerable Plaque - Definitions*
9:15 am – 9:35 am	Dr. Valentin Fuster - *Atherothrombosis and the High Risk Plaque*
9:40 am - 10:00 am	Break
10:05 am - 10:25 am	Dr. Richard Lee - *Biomechanical Properties of Vulnerable Plaque and Functional Genomics*
10:30 am - 10:50 am	Dr. Mark Rekhter - *Animal Models of Atherosclerosis and Plaque Rupture*
10:55 am - 12:00 pm	Controversies - *What is the Definition of a Vulnerable Plaque? What can be Accomplished with Animal Models?*
12:00 am - 1:00 pm	Lunch Break
Afternoon Session:	***Methods of Detection of Vulnerable Plaque***

1:00 pm – 1:05 pm	Chair, Dr. Valentin Fuster - Introduction
	1. Non-Invasive Detection of Vulnerable Plaque
1:10 pm – 1:20 pm	a) Dr. Chris O'Donnell-*The Epidemiology of Pre-Clinical Forms of Atherosclerosis: The Framingham Heart Study*
1:25 pm - 1:35 pm	b) Dr. Bruce Wasserman - *MRI for Vulnerable Plaque*
1:40 pm - 1:50 pm	c) Dr. Zahi Fayad - *MRI for Vulnerable Plaque*
1:55 pm - 2:05 pm	d) Dr. Thomas Hatsukami - *MRI for Carotid Vulnerable Plaque*
2:10 pm – 2:50 pm	e) Dr. John Rumberger - *EBCT for Vulnerable Plaque*
2:55 pm - 3:05 pm	f) Dr. Thomas Brady - *MDCT for Vulnerable Plaque*
3:10 pm - 3:20 pm	g) Dr. Bill Strauss - *Nuclear Methods for Vulnerable Plaque*
	2. Invasive Detection of Vulnerable Plaque
3:25 pm - 3:35 pm	a) Dr. E. Murat Tuzcu - *The Role of IVUS and Elastography*
3:40 pm - 3:50 pm	b) Dr. Gary Tearney - *Optical Coherence Tomography (OCT)*
3:55 pm - 4:05 pm	c) Dr. James Muller -*Near-IR Reflectance and Raman Spectroscopy*
4:10 pm - 4:20 pm	d) Dr. Scott Kinlay - *Intravascular MRI*
4:20 pm - 4:30 pm	e) Dr. Ward Casscells - *The Role of Temperature and pH Measurements in the Detection of Vulnerable Plaque*
4:35 pm – 4:45 pm	f) Dr. Takanobu Tomaru - *Angioscopic and Pathological Study on the Vulnerable Plaque and Stabilization of the Plaque by Bezafibrate*
4:50 pm - 5:05 pm	g) Dr. Scott Gazelle - *Cost-Effectiveness of Detection and Treatment of Vulnerable Plaque*
5:10 pm - 6:00 pm	Controversy - *Is There a Need for Methods (Non-Invasive, Including EBCT and/or Invasive) to Detect Vulnerable Plaque?*

Friday Evening Dinner

Harvard Faculty Club
20 Quincy Street
Cambridge, MA

7:30 pm Reception

8:00 pm Dinner and presentation of the
CIMIT Vulnerable Plaque Research Achievement Award

Saturday, October 6th

7:30 am - 7:55 am Continental Breakfast

Morning Session ***Treatment of Vulnerable Plaque***

8:00 am - 8:15 am Chair, Dr. Peter Libby - Introduction

Local Treatment of Vulnerable Plaque

8:20 am - 8:30 am Dr. Bernhard Meier

8:35 am - 8:45am Dr. Antoine Lafont

8:50 am - 9:05 am Dr. Robert Schwartz

9:10 am – 10:30 Experience with Drug-eluting Stents -

A New Possibility for Vulnerable Plaque Therapy

Dr. Mary Russell – *TAXUS – Paclitaxel-eluting Stents: From Bench to Bedside*
Dr. Emerson Perin –*Results of the Ravel Study, and Progress of The Sirius Study*
Dr. Alan Heldman – *Drug-eluting Stent Findings, and Results of the Aspect Study*

Systemic Treatment of Vulnerable Plaque

10:30 am - 10:40 am Dr. Andrew Selwyn - *Stabilization of Vulnerable Plaque by Lipid Lowering*

10:45 am – 10:55 am Dr. Mike Dunn - *Antibiotics*

11:00 am – 11:25 am	Controversy - *What is the Role of Local Versus Systemic Treatment for Vulnerable Plaque?*
11:30 am - 11:40 am	Dr. Mort Naghavi - *The VulnerablePlaque.Org Website*
	Future Research Needs
11:45 am - 12:15pm	1. Basic Science - Dr. Peter Libby
12:20 pm - 12:35 pm	2. Diagnosis and Therapy - Dr. James Muller

Task 2: Detection of Vulnerable Plaque using Optical Coherence Tomography
Principal Investigator: Brett Bouma, PhD, MGH

The specific goal of this year's research was to apply optical coherence tomography (OCT) for monitoring plaque development and response to systemic therapy in an animal model for atherosclerosis. Prior to this work, no longitudinal studies have been conducted within individual animals. The advantage of OCT is that cross-sectional images with a resolution approaching that of histopathology can be obtained *in vivo* (without sacrifice) so that disease can be monitored over time in individual animals. In order to make longitudinal studies with OCT practical, several technical issues have been addressed including imaging in the presence of blood, avoiding motion artifacts and providing sufficient resolution for plaque characterization.

Atherosclerotic disease in rabbits was generated using a high-cholesterol diet and focal balloon injury in the aorta and iliac arteries. The goal of the study was to monitor plaque response to diet modification and systemic therapy. Balloon injury was performed at the initiation of high-cholesterol diet. The first imaging time point was at 4 months. At that time, the rabbits were divided into separate arms of the study including diet modification and systemic therapy. The final imaging time point was intended to identify plaque response. The primary technical challenges addressed in this work were associated with the *in vivo* imaging. Balloon injury was performed using femoral access. OCT images of the rabbit iliac arteries and aorta were obtained by insertion of the imaging catheter through the carotid artery.

A second goal of the research was to investigate the ability of OCT to characterize plaque composition. As there is no existing gold-standard for plaque characterization *in vivo*, the team has conducted an extensive *in vitro* study.

Key Results: This past year the team has advanced the capabilities of OCT for imaging *in vivo* by resolving three key technical issues. First, the team has developed methods for displacing blood from the iliac and aorta using balloon occlusion and saline flush. Second, the team has demonstrated a sufficient image acquisition rate to avoid motion artifact due to respiration and pulsatile blood flow. Finally, the team has demonstrated that characteristic features in plaques can be resolved using a catheter that provides a resolution of approximately 10 microns.

Using non-DoD funds, the team has processed and analyzed corresponding OCT-histology pairs from a large series of human cadaver specimens (n=127). They found that objective OCT criteria are highly sensitive and specific for the diagnosis of fibrous, fibrocalcific, and lipid-rich plaques. These results represent an important step in validating this new intravascular imaging modality and will provide a basis for the interpretation of OCT images obtained in future clinical studies.

The team has demonstrated that OCT imaging of the rabbit iliac and distal aorta can be performed in live animals without sacrifice. The images have demonstrated that OCT provides adequate resolution and contrast to identify focal lipid rich plaques and that the extent of disease can be quantified. At the first OCT imaging time point the team has uncovered two problems suggesting that the time scale of our study must be extended. First, the young age of the rabbits acquired for the study results in small vessel sizes. This has made vessel ligation a challenging problem following imaging. Failure to adequately ligate the carotid has resulted in the death of three rabbits. A second problem is that the frequency of observing localized lipid rich plaques in the iliac and distal aorta is less than anticipated based on our preliminary studies. Extending the timeframe of the study should lessen the impact of both of these problems. The team is therefore requesting a no-cost extension for a period of six months so that both imaging time points can be delayed in all remaining rabbits.

Specific Aim 1: To develop, optimize and apply OCT imaging for the detection of vulnerable atherosclerotic plaques. To accomplish this Specific Aim the following items will be addressed:

- To identify OCT morphologic features that distinguish vulnerable from stable plaques,
- To determine catheter and imaging characteristics in a porcine model, and
- Validation of a rabbit atherosclerosis model.

Progress:

Obstruction of view by blood

New catheters will be designed and constructed to reduce the obstruction of view by blood during OCT imaging. Experiments will be performed on five swine to test three different methods of improving vessel wall visualization: 1) selective saline purging, 2) proximal occlusion, and 3) eccentric catheter design. Selective saline purging requires smaller diameter guide catheters and careful positioning of their insertion relative to the area of interest. Infusion rates will be varied to determine the optimal infusion rate for the longest duration of unobstructed imaging. The team will also investigate the use of occlusion proximal to the imaging site as is used in angioscopy. To accomplish this, a balloon segment will be integrated into the OCT catheter proximal to the imaging transducer. The third new method for enhancing visualization of coronary vessels that the team will investigate is the use of an eccentric catheter. Our preliminary studies have shown that significant imaging depth of penetration into the vessel wall can be achieved with less than 500 microns of blood between the catheter and the vessel lumen. An eccentric catheter would place the imaging transducer closer to one sector of the lumen and might effectively be used to image vessel structure with reduced or no saline purge. Rotating the catheter sheath would allow imaging of other sectors of the artery. Collaborations with an intravascular ultrasound (IVUS) manufacturer (Boston Scientific Corporation) and guide wire manufacturer (Microspring Company, Inc.) have been initiated to allow us to design and construct catheters better suited for OCT imaging in the coronary arteries.

Motion artifacts
The researchers participating in this project are the inventors of the high-speed reference arm delay line necessary for high speed OCT imaging. Current galvanometric techniques only allow for a scan speed of 4 fps. Modifications to this delay line using other devices such as a resonant scanner or an acousto-optic modulator to scan the optical delay can be used. The team will also be working closely with a galvanometer manufacturer (Cambridge Technologies, Inc.) to design an optimized linearly scanning galvanometer, based on our specific optical requirements. One tradeoff for increasing the image acquisition rate is that as the scan speed increases, the sensitivity of the system decreases proportionally. The team has recently purchased a high power broadband source for the cardiology OCT system that should offset this decrease in sensitivity and allow for up to 4x higher speed imaging. The catheter, system electronics, and software will be updated to accommodate for this speed increase. Once this is accomplished, the new high speed OCT system will allow images to be obtained at 16 fps (500 angular by 250 radial pixels) or 32 fps (250 angular by 250 radial pixels). Performance of this system will be assessed in the five swine used for Specific Aim 1.1.

High-resolution imaging
A new high power, broad bandwidth fiber optic source will be constructed and the OCT system will be modified to allow for high resolution imaging (2 μm axial resolution). The team is currently collaborating with a laser manufacturer (Coherent) to make this high resolution imaging source portable and capable of being easily transported to the cardiovascular suite. Once constructed, this system will be tested for its ability to resolve macrophages and other sub-cellular features in cadaveric coronary artery plaques *ex vivo* and rabbit plaques *in vivo*.

Rabbit Studies
A three arm study will be performed in rabbits to prospectively determine the ability of OCT to predict vulnerable plaques and monitor plaque regression. Sixty (n=60) New Zealand White rabbits (2.5-3.0 kg) will be started on a high cholesterol diet (150g/day, 0.5% cholesterol, 7% peanut oil). Blood will be withdrawn from the left ear vein at days 0, two months, and five months to measure the total cholesterol and triglycerides of each rabbit. After one week, balloon injury of the aorta and iliac arteries will be performed. IM injection of ketamine (35mg/kg)/xylazine(7mg/kg) will be administered with local anesthesia (lidocaine) at the inguinal region. A 4F Fogarty embolectomy catheter will be introduced through the left iliac artery, advanced to the aortic arch, inflated, and advanced back through the iliac artery.

The rabbits will remain on the high cholesterol diet for two months. At the end of this time period, another blood draw will occur, and OCT imaging will be performed. IM injection of ketamine (35mg/kg)/xylazine(7mg/kg) will be administered with local anesthesia. A 6F introducer will be placed in the left iliac artery. A 0.014" guidewire will be advanced into the aorta. Under fluoroscopic guidance, the OCT catheter (3F) will be advanced through the introducer, over the guidewire and into the aorta. OCT imaging of the aorta and left iliac artery will be performed using anatomic landmarks for image registration.

Following the first OCT imaging series, a lipid-lowering agent (e.g. Lipitor) will be administered to the rabbits in Arm 1. The high cholesterol diet will continue for three additional months, at which time all rabbits will be imaged by OCT using the previously described methods. The

rabbits in Arm 3 that were not given the lipid-lowering agent will have chemically induced plaque rupture immediately following final OCT imaging. All rabbits will be sacrificed and the OCT images and fluoroscopic images will be used to correlate histology with the OCT images. Blinded analysis of Arm 3 OCT images will determine the accuracy of OCT for predicting plaque rupture. Quantitative analysis of Arm 1 vs. Arm 2 (control) will allow assessment of the ability of OCT to monitor plaque regression.

Progress:

Identification of OCT morphologic features that distinguish vulnerable from stable plaques

Using non-DoD funds, a total of 127 atherosclerotic artery segments were obtained from 64 cadavers: 39 coronary arteries, 65 carotid arteries, and 23 aortas. OCT imaging was performed on each segment at 37°C. The specimens were sectioned and stained with Hematoxylin and Eosin or Movat's Pentachrome. A pathologist blinded to the OCT findings classified each plaque as fibrous (n=36), fibrocalcific (n=63), or lipid-rich (n=28). Using 15 randomly selected samples, OCT criteria for different plaque types were created. Fibrous plaques were characterized by the presence of a homogeneous, signal-rich region (Figure 1), calcific plaques by a sharply demarcated signal-poor region (Figure 2), and lipid-rich plaques by a signal-poor region with diffuse borders (Figure 3). These criteria were then applied in a prospective fashion to the remainder (n=112) of the OCT images by an investigator blinded to the pathologic diagnosis. Sensitivity and specificity were determined with 95% confidence intervals (CI). When prospectively applied to the test set, these OCT criteria were found to be 89% sensitive (CI: 0.78-0.96) and 98% specific (CI: 0.9-1.0) for fibrous plaques, 97% sensitive (CI: 0.83-1.0) and 94% specific (CI: 0.86-0.98) for fibrocalcific plaques, and 84% sensitive (CI: 0.64-0.96) and 94% specific (CI: 0.87-0.98) for lipid-rich atheromas. These results were submitted for oral presentation at the American Heart Association Annual Meeting (AHA 2001).

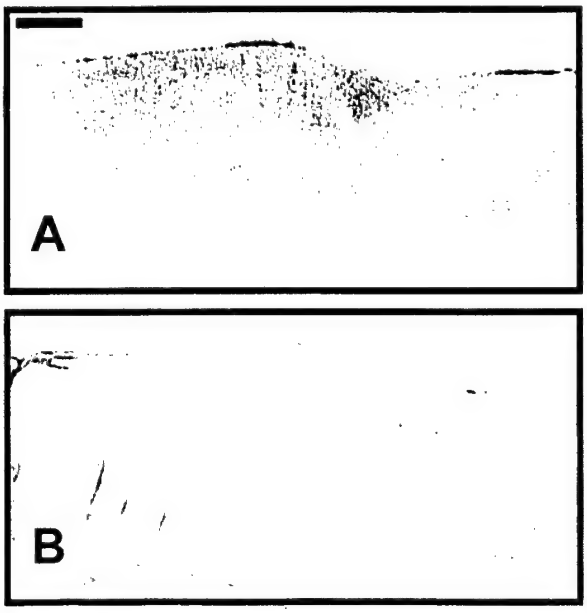


Figure 1. A. OCT image of fibrous plaque showing a homogeneous, signal-rich interior. B. Corresponding histology (H&E). Scale bar, 500 μm.

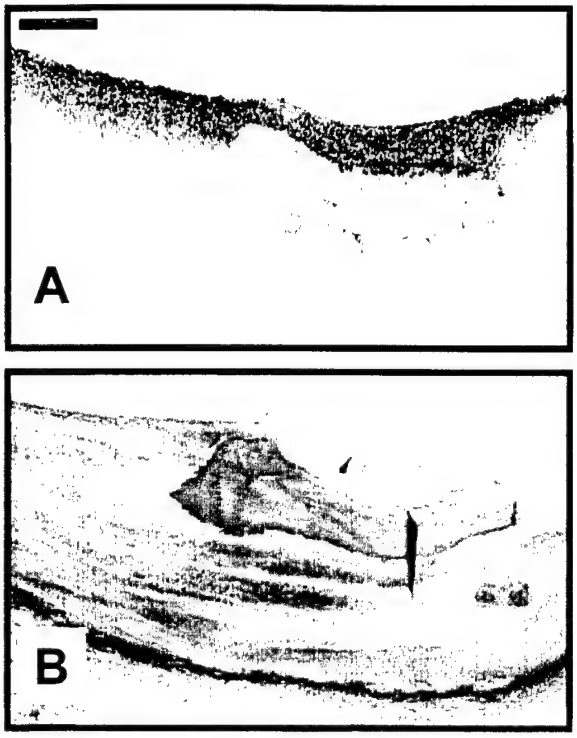


Figure 2. A. OCT image of fibrocalcific plaque showing a signal-poor interior with sharply delineated borders. B. Corresponding histology (Movat's Pentachrome). Scale bar, 500 μm.

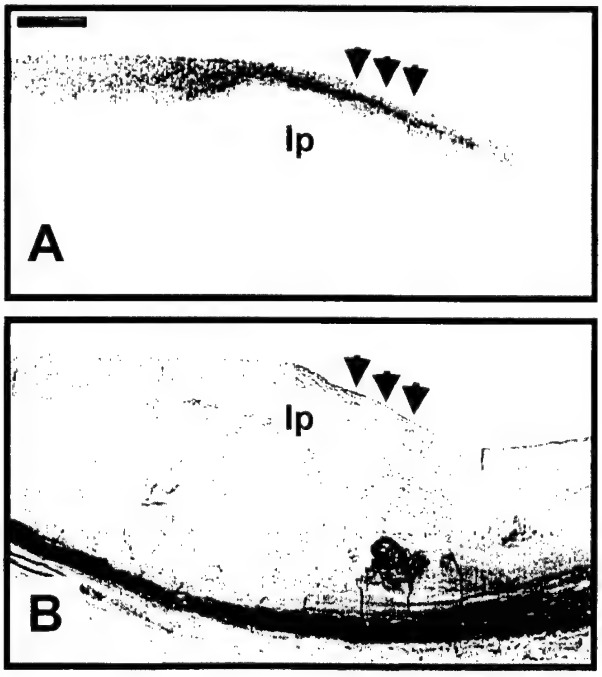


Figure 3. A. OCT image of lipid-rich plaque showing a signal-poor lipid pool (lp) with poorly delineated borders beneath a thin, reflective band, corresponding to the fibrous cap (arrows). B. Corresponding histology (Movat's Pentachrome). Scale bar, 500 μm.

Application of OCT to rabbit atherosclerotic plaque regression monitoring

During this past year 42 rabbits were obtained and started on a high cholesterol diet (Appendix A). The aorta and iliac arteries were injured following one week on the diet, in a staggered fashion (Figure 4). At the 4 month time point, the team initiated OCT imaging and have uncovered the problems described in the executive summary. Aorta and iliac vessels from the rabbits sacrificed in the control arm have been submitted for histologic evaluation. Pending this evaluation, the team proposes to extend the time point for OCT imaging by a period of two months to allow further plaque development. No additional funds are requested to cover this delay.

While imaging in the rabbit aorta, the OCT catheter was pulled longitudinally at a constant speed using a computer controlled motor. Images were acquired using different pull-back rates and evaluated for motion artifacts. At a pullback rate of 0.5 mm/s and an image acquisition rate of 4 frames per second, the vessel morphology could be sampled sufficiently to preclude significant motion blurring. Figure 4 presents a cross-sectional OCT image acquired in the aorta of a Watanabe rabbit ('XY Plane'). At the location of the blue cross-hair in the cross-sectional image, longitudinal images are computed from the pull-back data. Features such as side branches can be seen in the YZ plane (upper right) with minimal motion artifacts. In the XZ plane, the focal plaque cap is seen as a high-signal island protruding into the vessel lumen.

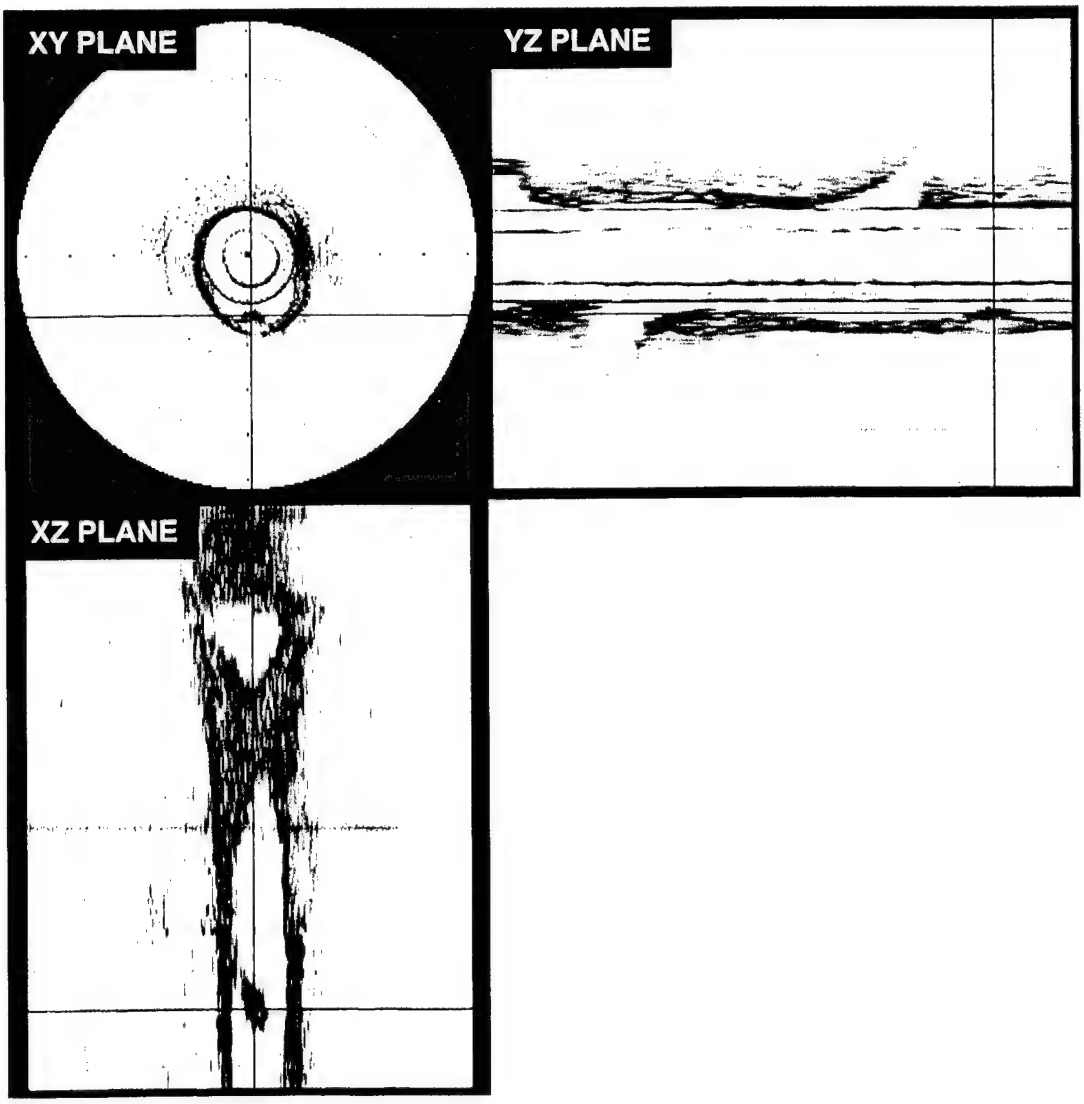


Figure 4. Transverse ('XY Plane') and longitudinal ('YZ Plane' and 'XZ Plane') cross-sections acquired in a living Watanabe rabbit aorta. Side branches of the aorta are seen in the YZ plane.

The third primary technical issue relevant to OCT characterization of atherosclerotic lesions is resolution. Autopsy studies indicate that the majority of lesions responsible for acute events consist of a large lipid pool with a thin (~65 μm) fibrous cap. The OCT catheter optical design was revised to provide higher transverse resolution. To assess the ability of the OCT catheter and system to characterize atherosclerotic lesions, imaging in a rabbit atherosclerotic model was performed. The image shown in Figure 5 was acquired in the aorta of a Watanabe rabbit that was fed a high-cholesterol diet for 2 months. Consistent with the findings of the *in vitro* study, lipid pools within the aorta wall present as signal poor regions (arrows in the Figure). In Figure 5B, a thin fibrous cap is seen as a layer of high signal overlying the lipid pool and has a thickness of approximately 20 μm.

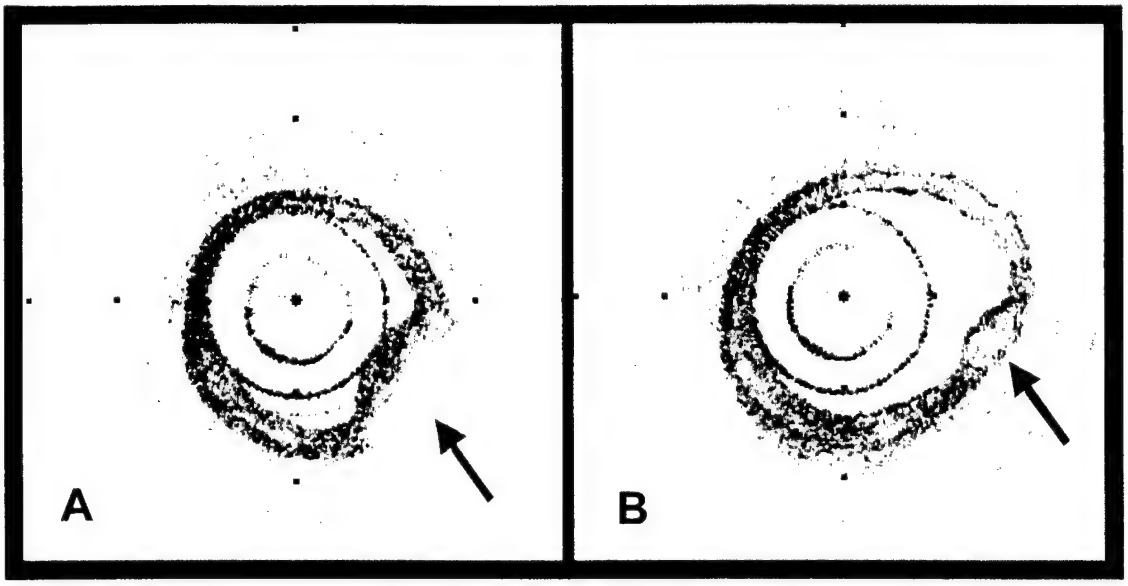


Figure 5. OCT images of aorta of Watanabe rabbit following 2 months high cholesterol diet. Low signal areas with dark overlying layer (arrows) denote lipid pools with fibrous caps.

Rabbit model of atherosclerosis

During the past quarter, preliminary studies to assess the validity of a rabbit atherosclerosis model were conducted. Six rabbits (3 New Zealand White and 3 Watanabe) were fed a high cholesterol diet (150g/day, 0.5% cholesterol, 7% peanut oil) for a period of 2 months. A balloon catheter was inserted into the proximal aorta and inflated. The OCT catheter was passed through the femoral artery using a guide wire under angioscopic guidance. Saline was introduced through the occluding balloon and OCT imaging was performed using a computer controlled pull-back device. Following imaging, the rabbits were sacrificed and the aorta was removed and fixed in saline. Histologic processing of the vessels is currently being performed. Although a comprehensive assessment of the plaque morphology will require histology, discrete lesions consisting of lipid and fibrous tissue were observed with OCT. Figure 6 depicts an OCT image acquired in the aorta of a New Zealand White rabbit.

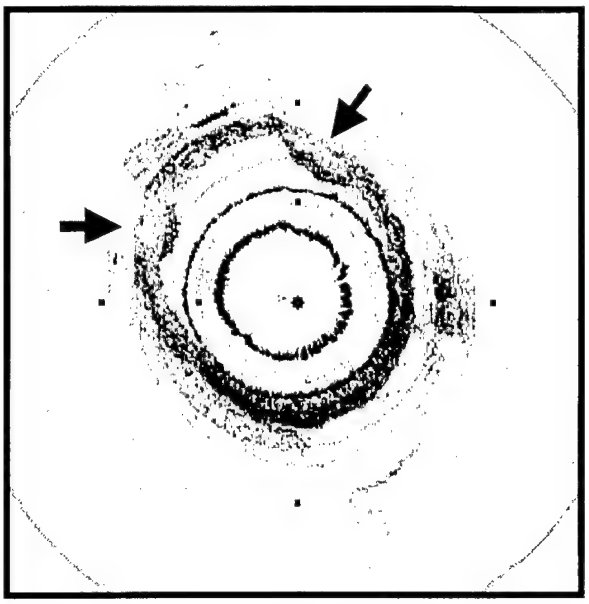


Figure 6. Atherosclerotic plaques in the aorta of a New Zealand White rabbit following 2 months of high cholesterol diet.

Plan: Results demonstrate that balloon occlusion in the proximal aorta with subsequent saline flushing is sufficient to permit clear OCT imaging over the entire length of the rabbit aorta. A pullback rate of 0.5 mm/s and an image acquisition rate of 4 frames per second are sufficient to avoid motion artifacts. The improved catheter optical design was shown to provide sufficient resolution to visualize fibrous caps with thickness of less than 20 μm. Based on these results, the entire research effort in the coming quarter will be devoted to the plaque progression and regression study.

3.3 STROKE

Stroke is the third leading cause of death and a major disability for over 4 million stroke survivors in the United States. There are 700,000 new stroke cases every year. Stroke has an economic impact of $30 billion a year.

The Acute Stroke Program at Partners HealthCare System has offered immediate evaluation and advanced emergency treatment over the last decade. Construction of a specially designed angiographic suite, which includes both state of the art MRI and digital subtraction angiographic (DSA) instruments, is nearing completion at the MGH and is expected to be operational by the Spring of 2001. This MRI/DSA unit will enable physicians to optimize delivery of existing stroke treatments, and, at the same time, provide CIMIT investigators with access to this facility for the rapid advancement of novel therapies in the management of acute stroke.

The overall goals of the CIMIT Stroke Program are: 1) to protect the brain from ischemic brain injury with brain hypothermia, 2) to treat clots that obstruct brain blood flow before they cause permanent brain injury; 3) to develop better means of non-invasively monitoring the brain for dangerously low blood flow and brain hemorrhage, 4) to develop non-human primate models to predict brain injury, and 5) to provide technology assessment to evaluate new therapies for the treatment of stroke.

Task 1: Acute Stroke Management – Neuro-Protection

Principal Investigator: Walter J. Koroshetz, MD, MGH and Albert S. Lee, MD, MGH

Brain cooling is the most potent neuroprotective strategy in the management of acute stroke. Its use as a neuroprotectant in ischemic brain injury is limited only by the lack of the means to achieve cerebral hypothermia in a rapid, safe fashion. Ongoing CIMIT funded research is focused on the development of novel interventive modalities to selectively cool the surface of the cerebral cortex to provide neuroprotection for stroke patients.

Specific Aim 1: Develop a means to quickly cool the brain cortex to afford neuroprotection.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Task 2: MRI Guided Rapid Laser Endovascular Photoacoustic Recanalization (LEPAR) for Hyperacute Stroke and Stroke Predictive Modeling

Principal Investigator: R. Gilberto Gonzalez, MD, PhD, MGH

The emphasis of this project remains the same as for the previous year. In the last year the team has been working in two main areas on this project: experiments with the arterial bypass device to supply blood to ischemic brain regions via the microcatheter (a potential therapeutic approach

for human stroke as well) and experiments with the survival macaque stroke model (needed to assess the longer term effect of potential therapies as well as to obtain control MRI data on stroke evolution in this new model). The arterial bypass device was improved via more bench top experiments and the purchase of a dual channel ultrasonic flow meter, allowing us to measure two flow streams at once (i.e. arterial blood and a second flow of dilutant solution, e.g. saline, or other solution mixed into the blood stream). Additional *in vivo* experiments utilizing this setup were partially successful.

The second focus area has been the survival macaque stroke model. So far the team has recovered one animal following the acute stroke procedures. This animal was recovered after 60 minutes of MCA occlusion, and was survived successfully for 24 hours. Observations at 24hrs after stroke induction showed that the animal appeared healthy with no noticeable deficits. This is an important advance as it demonstrates successful recovery from acute stroke, which is necessary for the long terms plans for this stroke model.

Key Results: This past year four animals were studied with the bypass setup: in two animals the correct flow rate was achieved and brain tissue in the lesion area was maintained viable for 60-90 minutes, while in one animal the flow rate was too low and the lesion tissue infarcted, while in one animal the flow rate was too high and hemorrhage resulted. These results identify the future directions for this part of the project: to improve flow stability using a better blood pump and to improve the means of obtaining the correct flow rate in each animal by measuring or calculating accurately the fluid pressure at the tip of the microcatheter within the brain.

Specific Aim 1: To demonstrate efficacy and tissue safety of the LEPAR device and to maximize patient safety by defining the irreversible brain injury probability by diffusion MRI in a primate model.

Progress: Work has proceeded on both the arterial bypass setup and on the survival stroke model. Work was done on development of the arterial bypass setup to better control the duration of the ischemia. Specifically the team has performed bench top experiments measuring pressure and flow rates through the micro-infusion catheters that are using for the macaque experiments. A critical issue when perfusing blood through the microcatheters to salvage occluded brain tissue is the pressure/flow relationship for a given catheter diameter. As shown by the plots below (Figure 1), for a given pressure, one can force about 4 times as much water through this particular catheter than blood, due to the viscosity differences of the two fluids. Moreover, for the smallest catheter (shown here) the pressure required to achieve even a flow rate of 1ml/min of blood is in excess of 1200mmHg. To obtain higher flow rates the team must use larger catheters (in which the team has limited freedom in this animal model, but more in humans), or dilute the blood. The team aims to focus on the latter approach, by lowering the hematocrit of blood in the bypass circuit by a small but well controlled amount in order to achieve higher flow rates.

With the recent purchase of a dual channel ultrasonic flow meter, the team can measure two flow streams at once, allowing continuous measurement of blood flow through the catheter, and an additional flow stream diluting the blood flow (e.g. using saline) in order to reduce viscosity of the blood flowing through the catheter. Additional *in vivo* experiments using this setup were

partially successful. In total 4 animals were studies with the bypass setup: in 2 animals the correct flow rate was achieved and brain tissue in the lesion area was maintained viable for 60-90 minutes, while in one animal the flow rate was too low and the lesion tissue infarcted, while in one animal the flow rate was too high and hemorrhage resulted. These results identify the future directions for this part of the project: to improve flow stability using a better blood pump and to improve the means of obtaining the correct flow rate in each animal by measuring or calculating accurately the fluid pressure at the tip of the microcatheter within the brain.

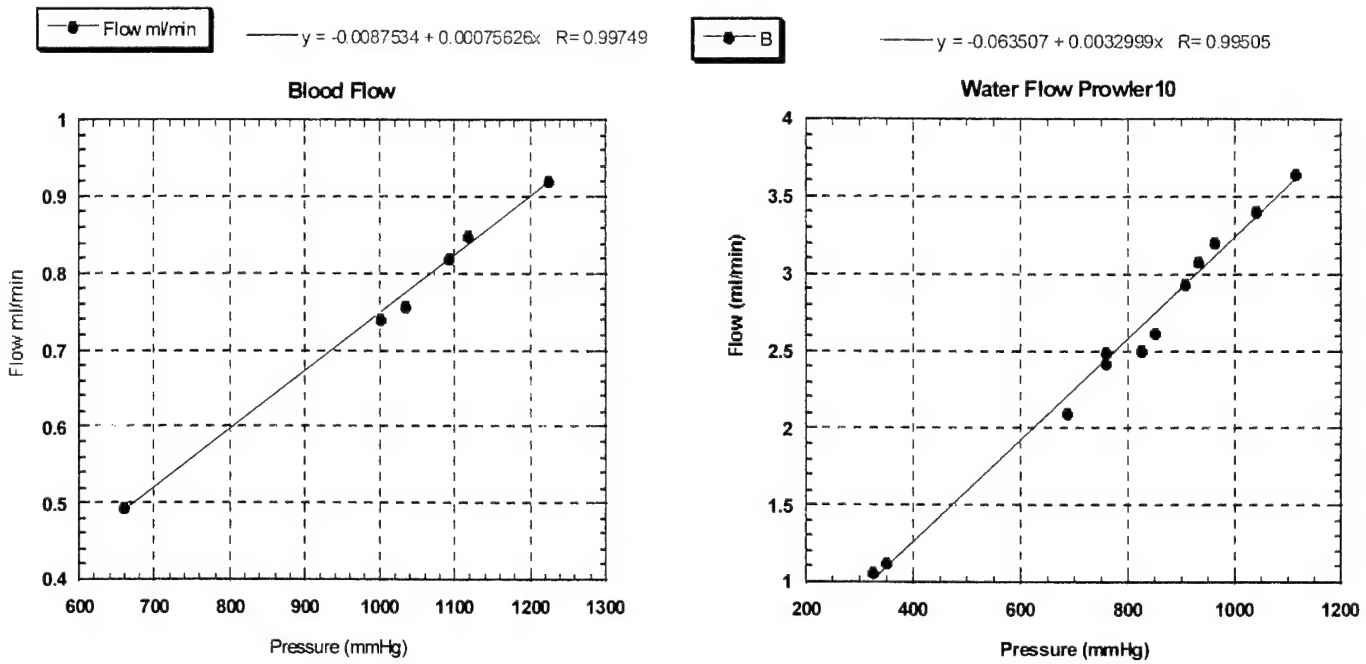


Figure 1: Pressure/Flow curves for the Prowler-10 micro-infusion catheter, use to occlude the vessels in o macaque stroke model. Left: curves for blood, Right: curves for water (saline).

In addition, the team has begun recovery macaque stroke experiment: so far the team has recovered one animal following the acute stroke procedures. This animal was recovered after 60 minutes of MCA occlusion, and was survived for 24 hours. Imaging data for this animal are shown in Figure 1. At 24 hours the animal's behavior was recorded and it was then sacrificed. The brain was removed immediately, placed in formalin, and scanned on a small-bore MRI scanner within 4 hours of death. MCA occlusion was verified angiographically after catheter placement. A region of perfusion deficit (delayed bolus arrival) was observed on the post-occlusion time-to-peak images but with only a slight focal rCBF reduction. No focal abnormalities were observed on the T2-weighted or ADC images. After reperfusion, PWI, ADC and T2-wt scans appeared normal. After 60 minutes of post-reperfusion scanning, the animal was recovered and returned to the primate center. At 24 hours, the animal appeared healthy with normal behavior and no noticeable motor deficits. The postmortem T2-wt and T1-wt scans acquired at 4 hrs after death show no focal abnormalities (previous experience with post-mortem brain MRI has shown good agreement between T2-wt imaging and histologically defined damage, although postmortem *diffusion* imaging is not useful). The data from this animal clearly demonstrates that the team can successfully recover animals from this endovascular occlusion

procedure. In this particular case however, it is likely that collateral circulation maintained tissue viability in the lesion area, in spite of a successful MCA occlusion.

This is an important advance as it demonstrates successful recovery from acute stroke, which is necessary for the long terms plans for this stroke model.

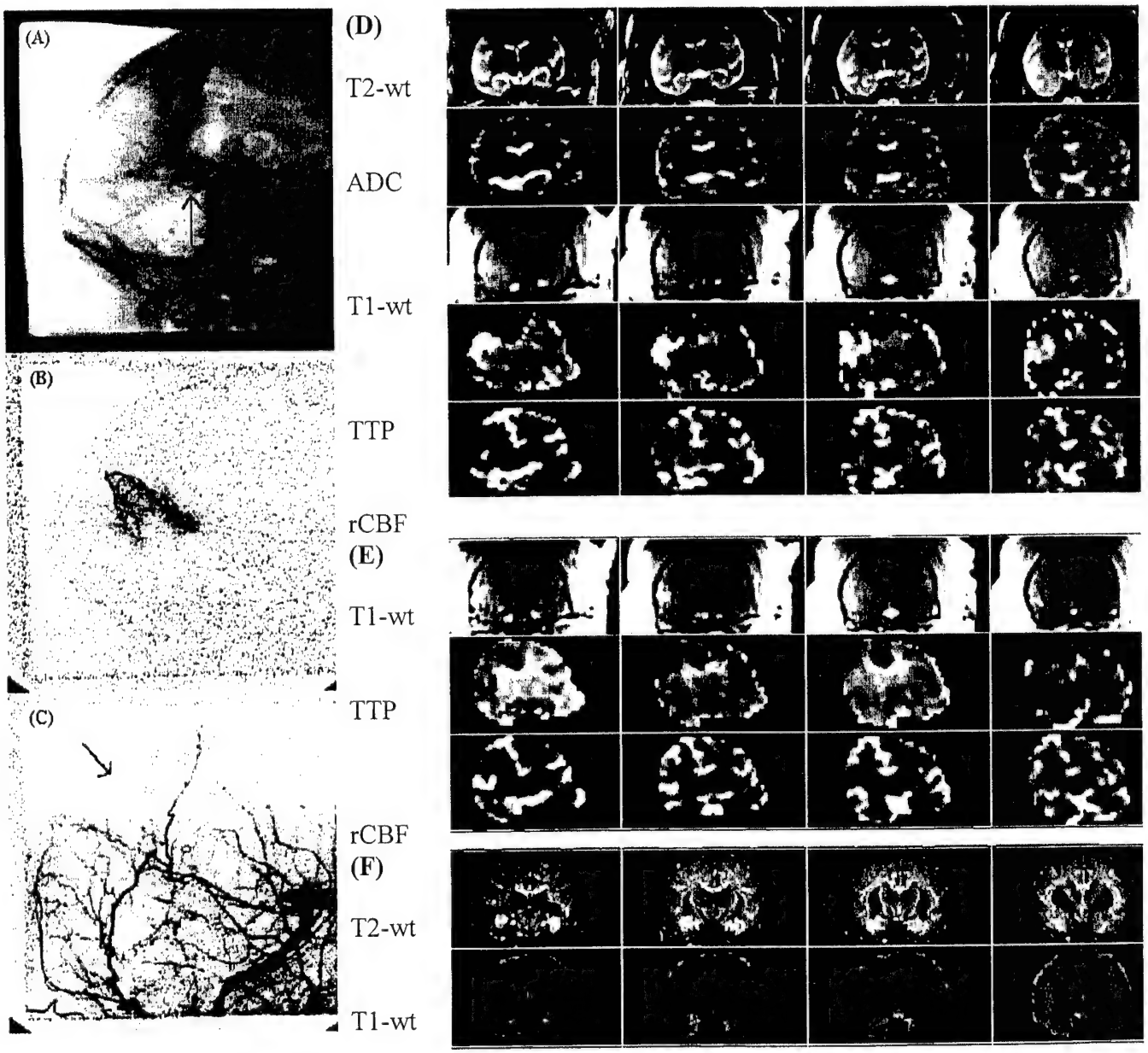


Figure 1. Recovery experiment. This animal received 60 minutes of MCA occlusion. (A) Lateral X-ray showing catheter tip. (B) Subtraction angiogram during contrast injection through catheter. (C) arterial angiogram showing wedge perfusion deficit. Panel (D) MRI at 40-50 minutes post *occlusion.* Panel (E) MRI at 15-25 minutes post *reperfusion.* Panel (F) MRI at 24hrs. (postmortem scans).

Plan: To continue our studies in the primate model to define irreversible brain injury using diffusion/perfusion MRI. The team aims to focus on achieving well controlled flow rates for blood in a variety of microcatheters, exploring different options for pumps and flow control and to continue with the survival experiments in the macaque stroke model.

Task 3: Optical Monitoring and Imaging of Stroke
Principal Investigator: Walter J. Koroshetz, MD, MGH and David A. Boas, PhD, MGH

The ischemic brain injury (stroke) program is targeted at developing better means of non-invasively monitoring brain for dangerously low blood flow and for brain hemorrhage; treating clots that obstruct brain blood flow before they cause permanent brain injury; protecting brain from ischemic brain injury with brain hypothermia. The optical imaging project is focused on developing a new imaging modality for continuous, bedside monitoring of brain perfusion. This year significant progress has been made on 1) developing 3D algorithms for solving the photon migration forward problem and inverse problem, 2) validating the quantitative accuracy of the optical method in piglets, and 3) developing a human subject research protocol.

Key Results: This year the CIMIT team fabricated a Continuous-Wave Imaging System with enough lasers and detectors to image stroke related effects over the entire brain surface. Also, the team developed accurate modeling of photon migration through the complex structure of the human head using Monte Carlo solutions of the radiative transport equation.

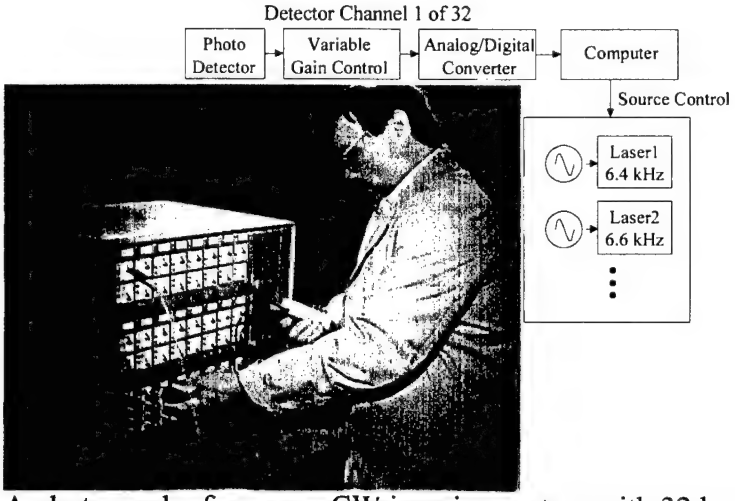
Specific Aim 1: To finish the construction and testing of the 3rd generation CW instrument and the 1st generation RF instrument.

Instrumentation and Algorithms
The endpoint of this aim is to develop quantitative 3D reconstruction algorithms and instrumentation to enable imaging over the entire adult human head. This is a challenging endpoint requiring multiple parallel investigations. The team will continue the investigation of constrained image reconstructions in order to exploit prior information to reduce the number of unknowns in the inverse problem. Year 3 will focus mainly on the exploitation of MRI anatomical information in the optical reconstruction. The team will work to better understand the effects of the layered structure of the head and then correspondingly optimize the reconstruction algorithms.

Progress: The team has made progress on 4 tasks: 1) Construction of a continuous-wave imaging system with 32 lasers and 32 detectors; 2) accurate modeling of photon migration in the adult human head; and 3) flattening the image sensitivity profile to make it spatially uniform.

Continuous-Wave Imaging System
Our continuous-wave (CW) DOT imaging system has 32 lasers (intensities driven at 32 different frequencies) and 64 detectors (see Figure 1). At present, the 32 lasers are divided into 10 lasers at 690 nm and 10 at 830 nm, with the remaining 12 split between 2 optical multiplexers each with 6 lasers (wavelengths of 685, 750, 808, 830, 906 and 980 nm, described in more detail below). The 10 – 690 nm lasers and the 10 –830 lasers go to fixed positions on the breast while

the 2 optical multiplexers switch the 6 colors of light between 300 positions on the human head. The detectors are avalanche photodiodes (APD's, Hamamatsu C5460-01). A master clock generates the 32 distinct frequencies between 6.4 kHz and 12.6 kHz in approximately 200 Hz steps. These frequencies are then used to drive the individual lasers with current stabilized square-wave modulation. Following each APD module is a bandpass filter, cut-on frequency of ~500 Hz to reduce 1/f noise and the 60 Hz room light signal, and a cut-off frequency of ~16 kHz to reduce the third harmonics of the square-wave signals. After the bandpass filter is a programmable gain stage to match the signal levels with the acquisition level on the analog-to-digital converter within the computer. Each detector is digitized at ~45 kHz and the individual source signals are then obtained by use of a digital bandpass filter - for example, a discrete Fourier transform or an infinite-impulse-response filter.



A photograph of our new CW imaging system with 32 lasers and 32 detectors.

Plan: Project completed.

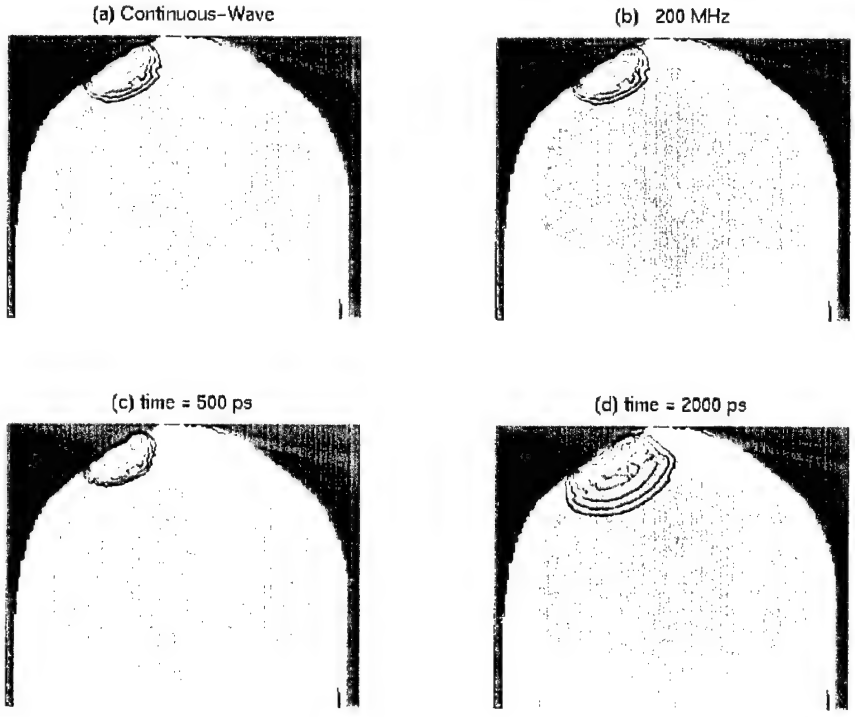
Specific Aim 2: Develop quantitative 3D reconstruction algorithims.

Progress:

Monte Carlo Modeling of Photon Migration in the Human Head

Photon migration theory models the propagation of light through tissue, and enables the extraction of information about the tissue from measurements of remitted diffuse light. By using Monte Carlo techniques the team can simulate the propagation of photons through tissue and obtain a spatial sensitivity. In such simulations, individual photon trajectories are traced by sampling appropriate probability distributions for scattering events. After 10^6-10^8 photons are traced, quantities such as the fraction of photons reaching a particular detector or the spatial sampling of the tissue can be determined. In our Monte Carlo model, the tissue is a three-dimensional volume with optical properties assigned to each voxel and source and detectors can be placed at any voxel. Photons are propagated until they exit the tissue and the total pathlength in each tissue type is recorded for all photons reaching a detector. Only recently has the computational power become widely available for the practical use of Monte Carlo simulations (one simulation typically takes 10-20 hours on current high-end desktop systems).

Figure 6 shows an anatomical MRI of a human head, segmented into five tissue types (air, scalp, skull, cerebral spinal fluid, and gray/white matter, with a contour overlay indicating the photon migration spatial sensitivity profile for (a) continuous-wave, (b) 200 MHz modulation, and (c,d) pulsed measurements. One contour line is shown for each half order of magnitude (10 dB) signal loss, and the contours end after 3 orders of magnitude in loss (60 dB) relative to the peak value for each image. For the 3D Monte Carlo simulation, the team assumed that $\mu_s' = 10$ cm^{-1} and $\mu_a=0.4$ cm^{-1} for the scalp and skull, $\mu_s' = 0.1$ cm^{-1} and $\mu_a=0.01$ cm^{-1} for the CSF, and $\mu_s' = 12.5$ cm^{-1} and $\mu_a=0.25$ cm^{-1} for the gray/white matter. Note how the contours extend several millimeters into the brain tissue, indicating sensitivity to changes in cortical optical properties. The depth penetration difference between the continuous-wave and 200 MHz measurements is difficult discern. A ratio of the two sensitivity profiles (not shown) shows that the 200 MHz profile is shifted slightly towards the surface. The time-domain sensitivity profiles are encouraging as they show that measurements made at longer delay times are able to penetrate significantly deeper into the brain tissue.



Photon sensitivity functions for a single source-detector pair (see text for details).

Image Artifact Reduction

The potential for image artifacts created by Tikhonov regularization methods popularly used in inverse solutions to the diffuse optical tomography problem has been tested. In particular it has been found that there are ringing artifacts of about ~10% the peak magnitude of the point spread function. A potential solution involves a two step image reconstruction process in which an initial image is reconstructed with additional noise and subsequently filtered using a Gaussian smoothing kernel. Preliminary results suggest a 10-fold reduction in artifact intensity. These results are outlined below.

Methods:
Simulations were based on a 1 dimensional array of interleaved sources and detectors arranged for reflectance imaging on a semi-infinite slab. The Rytov approximation is used and expressed as **y=Ax** by making the following associations:

$$y_i = \ln\left[\frac{\Phi(\mathbf{r}_{s,i}, \mathbf{r}_{d,i})}{\Phi_o(\mathbf{r}_{s,i}, \mathbf{r}_{d,i})}\right], \qquad A_{i,j} = -\frac{vh^3}{D_o}\frac{G(\mathbf{r}_{s,i}, \mathbf{r}_j)G(\mathbf{r}_j, \mathbf{r}_{d,i})}{G(\mathbf{r}_{s,i}, \mathbf{r}_{d,i})} \quad \text{and} \quad x_j = \partial\mu_a{}^j$$

The inverse problem, with Tikhonov regularization, is expressed as minimizing the following objective function:

$$\min\left\{\|\mathbf{y}_{meas} - \mathbf{Ax}\|_2^2 + \lambda \|\mathbf{x}\|_2^2\right\}, \qquad (6)$$

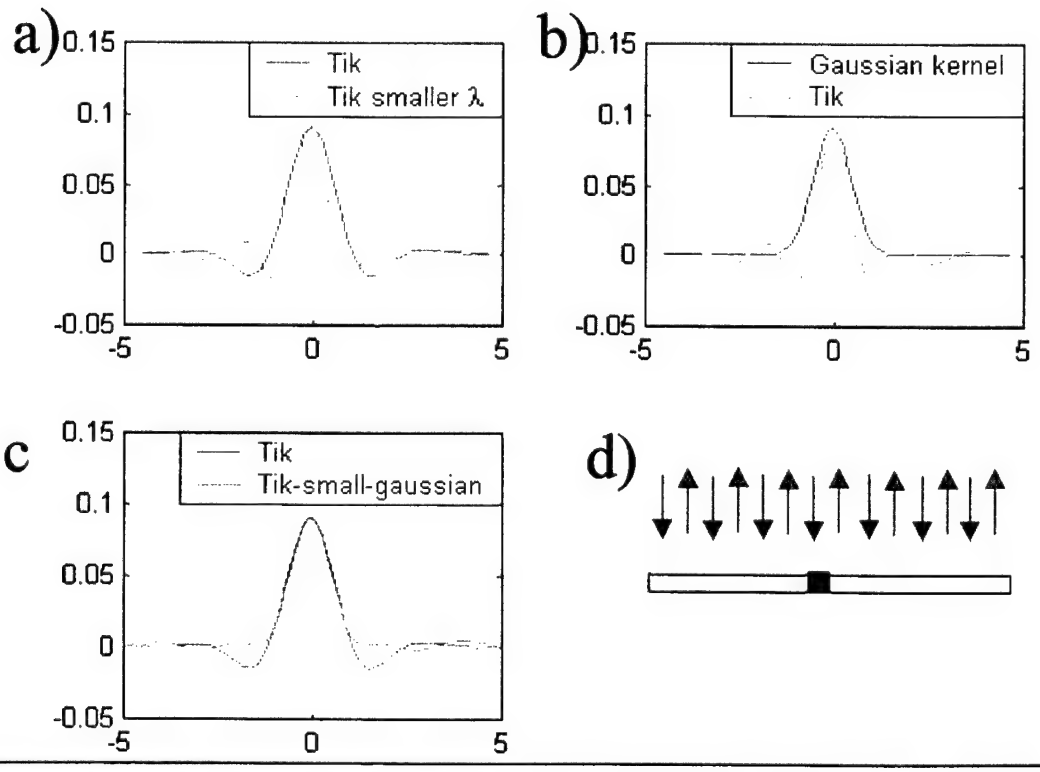
The equivalent solution can be obtained using the (Tikhonov regularized) Moore-Penrose generalized inverse,

$$\mathbf{A}_\lambda^{\#} = \mathbf{A}^T(\mathbf{A}^T\mathbf{A} + \lambda I)^{-1} \qquad \& \qquad x = \mathbf{A}_\lambda^{\#} y_{meas} \quad (7)$$

where I is the identity matrix. The value of λ is determined by imaging performance criteria. For example, a useful method called L-curve analysis determines the optimal lambda based on variance in the measurements and variance in the images.

A test image was created from $\mathbf{x}=\mathbf{A}^{\#}{}_\lambda\mathbf{A}\mathbf{x}^e$, where $\mathbf{x}^e$ was a single voxel point image with magnitude of 1. An example of this is shown in Figure 1a. Note that there are side lobes to the reconstructed image. These side lobes are ~10% the magnitude of the peak image intensity. The results for two different values of $\lambda$are shown.

The artifacts suggest an approach to avoiding them. If the narrower point spread function were averaged using a Gaussian smoothing kernel with width such that the resulting PSF was as broad as the larger PSF, then perhaps the ringing will be reduced. The team has implemented this, creating a Gaussian smoothing matrix G. The shape of the G function is shown in Figure 1 b. The final result is shown in Figure 1c. Note that the ringing artifacts have been reduced by an order of magnitude. Preliminary noise studies show that this procedure does not increase the noise when the initial small λPSF has a FWHM larger than 60% of the larger PSF that is matched by the Gaussian smoothing. This is being explored further using a two parameter L-curve analysis to simultaneously optimize both λ and the Gaussian FWHM.



a) The reconstructed point spread function(PSF) for two regularization constants. The ringing artifacts are ~10% peak height. b) A Gaussian smooth kernel and the PSF for the smaller value of λ. c) The PSF for the larger value of lamda and the improved PSF achieved by the combination of the smaller l PSF smoothed by the Gaussian kernel. d) Schematic of measurement geometry and the 1dimensional volume used to evaluate the PSF's.

Plan: In the future, the

activation conditions. It is expected that this instrumentation will provide accurate quantification of arterial and venous oxygen saturation during a baseline state. Furthermore, it is expected that this instrument will provide accurate images of brain activation, equivalent to the hemodynamic changes expected during a focal ischemia. Next year, it is also planned to develop time-domain instrumentation which is expected to provide quantitatively accurate information of baseline cerebral oxygenation and blood volume.

Specific Aim 3: To cross–validate the sensitivity and quantitative accuracy of the optical measurements with structural and functional information obtained with MRI, and to obtain preliminary results for "spin-off" projects.

Progress: The work this year has shown that the Photon Migration Lab's optical instrumentation can be used to measure venous oxygen saturation in muscle based on respiration-induced modulation of the venous blood volume. The plan is to extend this measurement methodology to the brain. Once accomplished, this will provide exquisitely sensitive measures of cerebral ischemia as indicated early by a reduction in venous oxygen saturation.

To compare $SvO_{2\text{-NIRS}}$ with the gold standard measurement of venous saturation from blood samples ($SvO_{2\text{-blood}}$), measurements were performed on three newborn piglets at the Massachusetts General Hospital. The piglets were anesthetized by inhalation of 3-4% Isofluorane

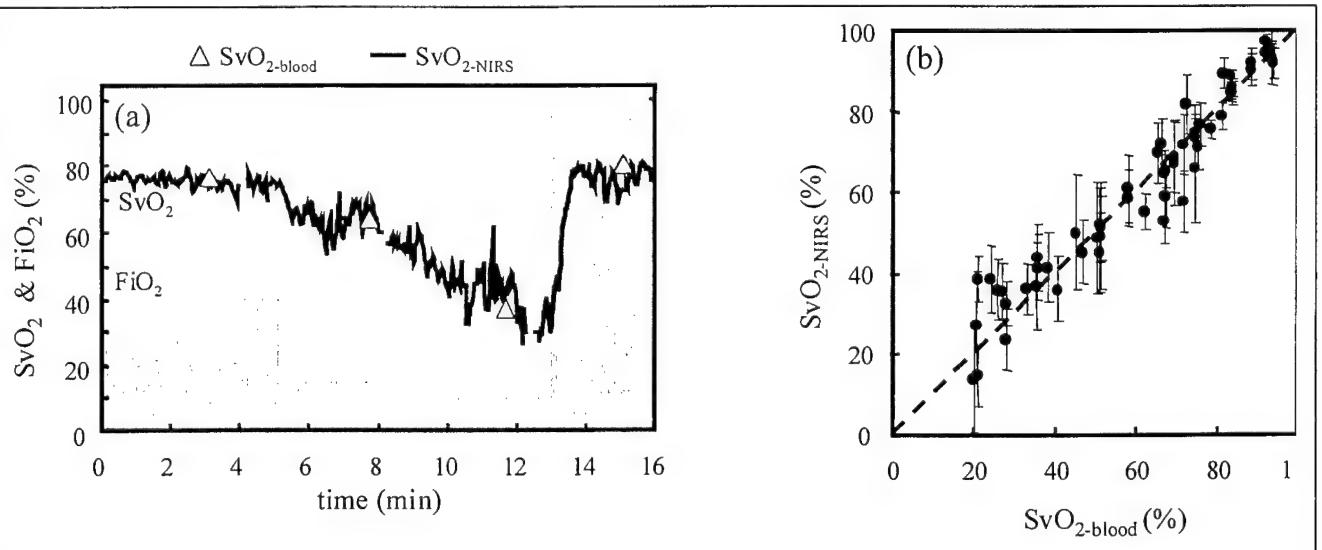


Figure 7. Comparison of $SvO_{2\text{-NIRS}}$ and $SvO_{2\text{-blood}}$ in the piglet study. Panel (a) comparison between the continuous measurement of $SvO_{2\text{-NIRS}}$, and the discontinuous measurements of $SvO_{2\text{-blood}}$ during a FiO_2 cycle in piglet 1. The values of FiO_2 (%, left *y* axis) during the experiment are indicated by the

administered by means of a breathing mask applied to the piglets' snouts. The animals were not mechanically ventilated and they breathed freely throughout the experiment. A femoral cut-down into the left inferior femoral vein was performed, to insert a catheter for periodic blood sampling. The femoral venous blood samples were run through a commercial blood gas analyzer to obtain invasive readings of the venous saturation ($SvO_{2\text{-blood}}$). An optical probe was located on the right (non-catheterized) hind leg. The protocol consisted of varying the femoral venous saturation over the approximate range 20-95% by modulating the volume fraction of oxygen inspired by the piglet (FiO_2) over the range 10-100%. Figure 7(a) compares the measurements of $SvO_{2\text{-NIRS}}$ on

the leg of piglet 1 during the first FiO_2 cycle with the invasive measurement of venous saturation on femoral vein blood samples ($SvO_{2\text{-blood}}$). Figure 7(b) plots the results of $SvO_{2\text{-NIRS}}$ on the legs of the three piglets against $SvO_{2\text{-blood}}$. The error bars are the standard deviations computed from the data collected during 80-sec intervals. $SvO_{2\text{-NIRS}}$ and $SvO_{2\text{-blood}}$ agree well over the whole range of venous saturation considered (20-95%). The two measurements showed an average difference of 1.0%, and a standard deviation of the difference of 5.8%.

Plan: The plan is to extend these measures of hemoglobin oxygen saturation to the brain. The effect of the complex structure of the brain needs to be considered. The Monte Carlo method described above will allow the exploration of the effect of the complex structure on the accuracy of such measurements. Furthermore, the planned development of a time-domain instrument and its application to cerebral oximetry will be tested and compared against the current continuous-wave instrumentation in this piglet model.

Specific Aim 4: To demonstrate the clinical utility of diffuse optical tomography (DOT) for continuous monitoring and diagnosis of cerebral hemorrhage and stroke.

The endpoint of our project is to demonstrate the clinical utility of DOT for continuous monitoring and diagnosis of cerebral hemorrhage and stroke. A pilot study in patients will be developed to determine the sensitivity to these pathologies and to identify practical problems associated with transferring the optical technology from "the bench to the bedside." An important feature of the study for next year will be to correlate anatomical MRI images of patients with the optical measurements, and use the combined data to extract more information concerning the stroke and the optical sensitivity to it.

Progress: Work is progressing on obtaining approval for human subject research.

Plan: Obtain human subject research approval.

Task 4: Neuronal Injury and Neuroprotection in Epilepsy: Proton Beam Radiation for Intractable Epilepsy

Principal Investigator: Jonathan L. Brisman, MD, MGH

A new model of proton beam radiosurgery (stereotactically focused irradiation) of the rat hippocampus has been developed. This model appears to be robust with brain necrosis evidenced reliably after a 3 month latency using doses of 90 Cobalt Gray Equivalents (CGE) or greater. This unilateral necrosis has been shown to correlate with increased T2 signal on MRI, decreased ability to perform the Morris Water Maze and the diminution of excitatory post-synaptic potentials and granule cell field spike obtained using *in vivo* microelectrode recordings. Positive alterations in heat shock protein, parvalbumin, calbindin and calmodulin have been detected. Upregulation of heat shock protein at non-necrotic doses may be important in explaining why low-dose irradiation reduces seizure activity in humans. These findings have been presented orally at the Congress of Neurological Surgeons Annual Meeting, September 2000, at the Spring Hippocampal Research Conference in spring 2000 and presented at the national Radiology Conference in fall 2000.

Two additional time points after irradiation have been employed to further study the time course of irradiation effects on the rat brain. Twelve animals have been studied five hours after irradiation and eighteen animals ten months after irradiation. The animals studied at the five hour point show apoptotic cell death in the irradiated hippocampus in a dose-dependent fashion. The ten month animals appear to show physiologic changes even at the lower doses used; histologic analysis has not yet been done.

A cohort of 40 animals has been irradiated after receiving pilocarpine status epilepticus. These animals have been analyzed physiologically and their brains stained for histologic and immunochemical analysis. In addition to the immunochemistry previously used in the normal rat brain irradiation study, a "Timm Stain," that typically shows axonal sprouting after pilocarpine seizures, was employed to determine whether irradiation has any effect on this neuronal response to status epilepticus. Preliminary results suggest that the pattern is altered with the higher doses employed.

Specific Aim 1: To characterize the histologic and electrophysiologic effects of proton beam irradiation in the normal rat brain.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 2: To determine whether proton beam radiation can alter the neurophysiology or anatomic changes in animals that have undergone 24 hours of perforant pathway stimulation.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Specific Aim 3: To determine the brain MRI appearance of rodents subjected to varying dosages of proton beam irradiation as well as rodents that have undergone 24 hours of perforant pathway stimulation.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Task 5: Measurement of Vascular Reactivity by Functional MRI in Cerebral Amyloid Angiopathy

Principal Investigator: Steven M. Greenberg, M.D., Ph.D.

In cerebral amyloid angiopathy (CAA), the β-amyloid peptide is deposited in small and medium-sized vessels of the leptomeninges and cerebral cortex. Amyloid replaces vascular smooth muscle, transforming vessels into rigid structures. In severe cases, this leads to rupture of the vessel wall and cerebral hemorrhage. Current diagnostic methods for CAA are limited to pathologic examination of the brain or radiographic demonstration of multiple hemorrhages in a characteristic distribution. Although CAA often affects a large number of cerebral vessels, whether the disease changes the flow characteristics of these vessels is unknown. Functional MRI (fMRI) can reliably asses human cerebral oxygenation, blood flow, and, with gadolinium injection, cerebral blood volume. The team is investigating two stimuli that reliably increase cerebral blood flow in humans: visual stimulation using a flashing checkerboard pattern and CO_2 inhalation. Because cerebral blood flow is intimately tied to oxygen demand, visual stimulation increases flow through the normal activation of neuronal activity, and the recording of such changes has been the cornerstone of fMRI. By increasing CO_2 in the blood, CO_2 inhalation results in dilation of cerebral vessels and increased cerebral perfusion. The team hypothesizes that changes in blood vessels secondary to amyloid deposition alter the degree to which vascular tone can respond to both increased neuronal activity and CO_2 inhalation. This project investigates whether fMRI can document a difference in the response of cerebrovascular tone to these two stimuli between patients and controls. These techniques may enable us not only to diagnose cerebral amyloid angiopathy before the first hemorrhagic stroke, but also to follow patients over time, assessing the progress of their disease and response to potential therapies.

Key Results: This year, four control patients were studied using fMRI. Data analysis revealed a robust response in blood flow to both visual stimulation and CO_2 inhalation.

Specific Aim 1: To develop a protocol for measuring cerebrovascular dilation in response to visual stimulation and 5% CO_2 inhalation in the elderly.

Progress: Cerebrovascular reactivity to visual stimulation has been measured in 4 control patients by examining the changes in blood oxygen level dependent (BOLD) signal and regional cerebral blood flow. MRI revealed increased regional cerebral blood flow responses to visual stimulation (Figure 1) in 3 patients and CO_2 inhalation in 2 patients (Figure 2). Two patients were not administered CO_2 and a fourth patient, in whom no blood flow response was measured during visual stimulation, likely closed her eyes during much of the MRI.

It has been difficult to successfully recruit CAA patients, as many have been ineligible due to cardiac or pulmonary exclusions. At the present time, the Massachusetts General Hospital Institutional Review Board (MGH IRB) is reviewing a modified version of this protocol that will allow the omission of the CO_2 challenge in CAA patients with cardiac or pulmonary contraindications so that they may be included in the study. This proposed amendment represents no change in currently approved methodology or techniques, and it will allow this study to proceed safely with more available subjects. The Department of Defense will receive formal notification of this amendment as soon as the MGH IRB has approved it.

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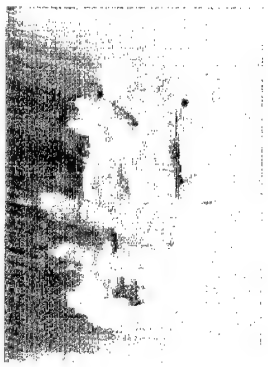

Figure 1. (WN) BOLD sequence from a 68-year-old control patient exposed to a visual stimulus. Areas colored red and orange demonstrate increased regional cerebral blood flow.

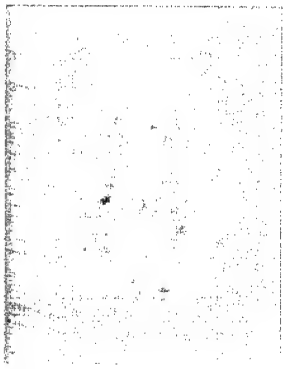

Figure 2. (FS) BOLD sequence from a 67-year-old control subject exposed to 5% CO_2 demonstrates more diffusely distributed changes in regional cerebral blood flow when compared to the BOLD response obtained with visual stimulation.

Plan: The team plans to study an additional control patient and five patients with CAA.

4.0 TECHNOLOGY ASSESSMENT AND OUTCOMES ANALYSIS PROGRAM

Principal Investigator: G. Scott Gazelle, MD, MPH, PhD

Recent advances in the life sciences and bio-engineering have resulted in a virtual explosion of exciting new medical technologies. These technologies have the potential to improve in healthcare in ways and at a rate heretofore considered impossible. However, modern healthcare is provided in an environment where costs are considerable and resources limited. Obtaining maximum impact from limited resources therefore requires that clinical impact be assessed before significant clinical data are available.

The CIMIT Technology Assessment and Outcomes Analysis Program was initially established in order to facilitate accurate and expeditious evaluation of new, minimally invasive technologies. Our initial goal was to develop a program fully integrated with all CIMIT research, clinical, education, and administrative activities. These initial goals have been accomplished, and the Program today is a central component of CIMIT's plans for the future.

The principal activities of the CIMIT Technology Assessment and Outcomes Analysis Program include decision analysis (DA), cost-effectiveness analysis (CEA), outcome analysis and healthcare policy research.

Key Results: This past year, the CIMIT team's principal research efforts have continued to focus in three principal areas: Stroke, Vulnerable Plaque, and the Operating Room of the Future Project.

Specific Aim 1: To focus resource allocation for the development of new minimally invasive technologies.

Specific Aim 2: To facilitate rapid and accurate assessment of effectiveness and cost-effectiveness.

Specific Aim 3: To demonstrate the value of these technologies to the public, physicians, payers, industry, and legislators in order to facilitate appropriate implementation.

Progress: The Program now includes 12 investigators with training and expertise in the core disciplines of biostatistics, epidemiology, economics, decision science, outcomes analysis and health care policy. Several clinical and research fellows also participate in specific research projects. Program members participate in the full spectrum of CIMIT research, clinical, educational, and administrative activities.

Stroke

Working with the Stroke CFA, the team developed a database with over 7000 patients treated for cerebrovascular disease at MGH or BWH during FY 94-99. This database provides the capability to answer questions concerning the costs and benefits of stroke treatment and will enable analysis of diagnostic and therapeutic interventions. An economic model was developed to predict stroke costs and outcomes, and investigate the benefits of early triage (based on diagnostic testing), as well as the role of comorbidities and socioeconomic status in determining

outcomes. A revised manuscript comparing the costs of acute ischemic stroke, across different stroke subtypes, has recently been resubmitted to *Stroke*. Another manuscript analyzing the costs of hemorrhagic stroke is nearing completion. An analysis of functional neuro-imaging has recently been initiated, as has an analysis on the impact of new technologies on the overall costs of stroke care.

Image-Guided Therapy
The team has investigated the cost-effectiveness of surgical resection of liver metastases from colorectal carcinoma, and the effect of diagnostic imaging in this setting. The first manuscript resulting from this work is now under review; several additional manuscripts are in the final stages of preparation. The analysis has now been expanded to investigate the benefits and appropriate role of image-guided, in-situ tumor ablation.

The team worked with Norm Nishioka to design and implement an analysis of the appropriate role and potential benefits of OCT in upper gastrointestinal disorders, and ALA-assisted endoscopy in Barrett's esophageal dysplasia. The study is ongoing, and a cost-effectiveness analysis has recently been completed. A manuscript describing the results of this analysis is currently under review.

A cost-effectiveness analysis of imaging and therapy in patients with pancreatic cancer has recently been completed. A complex decision analytic simulation model was developed and verified by comparing its predictions to actual clinical data. The results of this study were recently published in *Radiology*. This project grew out of the earlier pancreatic focus for the Cancer CFA.

Simulation
An analysis of medical simulation has recently been initiated. This involves an investigation of learning curve dynamics and their relationship to medical errors and training costs. Due to the lack of significant prior work in this field, the research is largely theoretical; however, progress has been rapid and the analysis has begun to establish a basis for determining the impact and value of medical simulation as a training tool in a variety of clinical settings.

Catheter-Based Interventions
A cost-effectiveness analysis of percutaneous abdominal aortic stent placement is underway. This involves a comparison of percutaneous stent placement to open surgery, and an assessment of the impact of this new technology on patient survival and costs, as well as the population-wide effects of changing treatment thresholds. A manuscript comparing the cost of open surgical and endovascular repair has recently been accepted for publication; a related paper is under review.

An assessment of quality of life in patients with menorrhagia is underway. The goals of this project are to assess health-related quality of life preferences, to further refine a new assessment instrument (the "binary gamble" method; initially developed by Johanna Bosch for use in peripheral vascular disease), and develop a decision model to evaluate the cost-effectiveness of minimally invasive therapies (e.g., uterine artery embolization).

Plan: The CIMIT Technology Assessment and Outcomes Analysis Program represents an evolution of the Program established three years ago. As a result of discussions with members of the Operations Group, and in view of changes in CIMIT over the years, the team has downsized and reshaped the Program to concentrate our research efforts in two major focus areas. Service, policy and administrative components of the Program will now be concentrated in a Program Core. The Technology Assessment and Outcomes Analysis Program will be an important component of ongoing CIMIT technology development efforts. The Program will help CIMIT focus resource allocation for the development of innovative technologies that can result in improved patient care; perform studies to assess the effectiveness, cost and cost-effectiveness of technologies under development; and demonstrate the value of these technologies to the public, physicians, payers, industry, and legislators in order to facilitate appropriate clinical implementation. Our overall goal is to help CIMIT determine and optimize the outcomes of its research efforts, and more generally to redefine the manner in which healthcare interventions are valued.

The Program is now divided into a Core and two major Outcomes Projects. The Program Core will: 1) assist the Operations Group with resource allocation decisions by performing preliminary analysis of technologies identified in requests for funding; 2) provide scientific direction, project coordination and administrative support to three major Outcomes Research Projects; and 3) provide consultation and guidance to CIMIT investigators and collaborators regarding issues such as project feasibility, study design, optimal endpoint determination and data analytic or statistical methods.

The Outcomes Projects were developed in response to the needs of three CIMIT major focus areas and are the result of ongoing collaborations. The models developed and research results form each of these projects will be a resource not only for CIMIT, but also for collaborators in government and industry.

In the Vulnerable Plaque Outcomes Program, the team will develop a comprehensive model of cardiovascular disease and therapy. The model will be used to evaluate the clinical effectiveness and economic impact of diagnostic and therapeutic interventions in coronary artery disease, ranging from screening, through non-invasive and invasive diagnostics, to local and/or systemic therapy. The team will examine specific diagnostic and therapeutic technologies such as optical coherence tomography, spectroscopy, ultrafast CT, and statin therapy. The model will also be used to identify relative effectiveness and/or cost thresholds that a new technology must meet in order to be a viable alternative to currently available technologies. The completed model can be used to help focus technology development and to answer policy-related questions regarding the benefits of specific technologies for individual patients and the population at large. It will serve as a useful tool for physicians, scientists, industry, payers and policy makers, all of whom must make difficult decisions concerning the development and implementation of these technologies.

In the Operating Room of the Future Outcomes Project, the team will develop, verify, and utilize a discrete event simulation model in order to evaluate all aspects of the OR of the Future project. The research will proceed from model development and validation through a careful assessment of the effect of each component technology or process on patient outcomes and system

efficiency. Our goal is to develop a comprehensive, robust and expandable model of the entire surgical suite, in order to better understand the optimal approach to integrating OR of the Future activities. Our analysis will focus on identifying the most effective new technologies and techniques as they are developed, in order to help guide resource allocation for further development and clinical implementation. The model will serve as a resource to those developing new OR technologies, as well as to those who must decide whether or not to deploy them.

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6.0 APPENDICES

APPENDIX A: CIMIT EDUCATION PROGRAM
Program Director: Reuben Saul Mezrich, MD, PhD, BWH

The Education Program was established to develop innovative methods of training and education in technology-based and interdisciplinary medical research and practice. The intent was to develop a program that would create novel approaches to training and education, integrated with all of CIMIT's research and clinical activities. The Program's principal activities in the past year included the development of simulation-based training tools, oversight of CIMIT's internal education Forum, and the integration of students into CIMIT's research activities. These activities were carried out in order to help CIMIT with the Specific Aims, below. Aspects of the Education Program, devoted to the Simulation Program and the Surgical Planning Lab, will be directly incorporated into these programs. Forum activities will continue as before, with greater emphasis on technology directed to medical care, in order to reflect a heightened interest in creating more technology/medical interactions.

Specific Aim 1: Develop leading edge technology-based systems for medical training.

Progress: This year, the team continued to assist the CIMIT Simulation Program to define specific procedures and refine technical requirements for the haptics and modeling components of its first simulation-trainer prototype. The Education Program has collaborated with the CIMIT Simulation Program and CIMIT Surgical Planning Lab to apply for funding as an NIH Bioengineering Research Program, which includes development of a second prototype system in conjunction with the CIMIT Surgical Planning Lab. The Education Program has collaborated with the Center for Medical Simulation to design and begin execution of a study to develop and validate assessment tools and training curriculum for cardiopulmonology. A partnership with the PASTUER program in patient-oriented research was set up to produce curriculum and experiments in simulation-based education in conjunction with the CIMIT Education Program and the Center for Medical Simulation.

Plan: To continue to incorporate the elements of the Education Program into the Simulation Program and the Surgical Planning Lab Program.

Specific Aim 2: Engage students in CIMIT research on a regular and formal basis.

Progress: The MIT Medical Innovation course is Co-Directed by Reuben Saul Mezrich MD, Ph.D. , Director of Technology at CIMIT and by John Guttag Ph.D., Chairman of Electrical Engineering and Computer Science (EECS) at MIT. The goal of this course is described in the MIT catalog:

> "*The goal of this subject is to provide opportunities for graduate students and others to identify research topics that combine engineering and medicine. Topics will be presented in two-week blocks. A physician will introduce each topic in the first week. The physician will describe his aspirations and then describe technical hurdles to be overcome to achieve the goals. These hurdles, or at least some aspects of them, will be assigned as homework*

assignments to groups of students. In the second week the students will present their ideas for attacking the research problems.

Tentative topics include extracting information from medical data, trauma and biosensors, percutaneous treatment of cardiac disease, image guided surgery, endoscopic and robotic coronary surgery, and cross-sectional cardiac imaging."

The course will be repeated in the Fall Semester, 2001.

Plan: Students from HST and other programs in MIT will continue to be integrated into CIMIT research activities and CIMIT faculty will continue to act as mentors to these students.

Specific Aim 3: Develop interdisciplinary forums for the exchanges of ideas.

Progress: See CIMIT Forum, below.

Plan: Continue the Forum, with constant adjustment of format and more aggressive recruitment of speakers to talk about implementation of new technology into medicine.

Specific Aim 4: Develop innovative, technology-based outreach programs.

Progress: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

Plan: Project completed. See Quarterly Progress Report January 1, 2001 through March 31, 2001.

CIMIT FORUM

TOPICS FROM CIMIT FORUM DURING LAST YEAR
(October 1, 2000 – September 30, 2001)

Medical Technology Needed for the Exploration of Space -- the NASA Perspective Julie Swain, MD, Acting Deputy Associate Administrator, NASA

Breath Analysis for Medical Diagnosis: Implementation of a New Technology Raanan Miller, Ph.D. Draper Laboratories (DL)

New Aspects of the FDA and HCFA Approval Process

"Implementation of least burdensome means"
John J. Smith, M.D., J.D. Director of Regulatory Affairs, CIMIT, MGH

"Evolution in FDA requirements for stent approval"

Richard Kuntz, M.D. Department of Medicine, Division of Clinical Biometrics, Brigham and Women's Hospital (BWH)

"Applying the HCRI/CDAC model to non-cardiac area" James Breitmeyer M.D., Ph.D. President and CEO, Harvard Clinical Research Institute

"Cost-effectiveness in stenting and radiation"
David Cohen, M.D., MSc Assistant Professor of Medicine, Harvard Medical School, Director of Interventional Cardiology Research, Beth Israel Deaconess Medical Center

CIMIT Program Review 3: Image Guided Therapy
Ferenc Jolesz, MD Diretor of MRI, Director of Image-Guided Therapy Program, BWH Kullervo Hynynen Ph.D., Head of Focussed Ultrasound Laboratory, BWH

Clinical Focus Session on Endoscopy: The Edges of Current Practice
David Carr-Locke, MD, Director of Endoscopy BWH

Security, Privacy and Confidentiality in Emerging Medical Applications
Barbara Beckerman, PhD, Oak Ridge National Laboratories

Robotics in Surgery
David Torchiana, MD, Chief of Cardiac Surgery, MGH

Volumetric Image Navigation via a Stereotactic Endoscope
Ramin Shahidi, PhD, Image Guidance Laboratory, Stanford University Medical School

CIMIT Program Review 4: Simulation
Steve Dawson, MD Director, CIMIT Simulation Program MGH, Stephan Cotin PhD Research Lead, CIMIT Simulation Program MGH, Jeff Cooper PhD Director, Center for Simulation, Director, Partners Biomedical Engineering, Micahl Berris Graduate Student MIT, Mark Ottensmeyer, Graduate Student, MIT

Building the Nano-Doc: An Overview of the Bioinstrumentation Lab at MIT
Ian Hunter, PhD Head of the BioInstrumentation Lab MIT, Sylvain Martel, Ph.D. Department of Mechanical Engineering, MIT John Madden, Ph.D. Department of Mechanical Engineering MIT

CIMIT Program Review 5: The Future of the Operating Room
David Rattner, MD, Assistant Chief of General Surgery, MGH, Keith Isaacson, MD, Vincent Obstetrics and Gynecology Service MGH, Clare Tempany, MD, Department of Radiology, BWH, Nathaniel M. Sims, MD, Division of Anesthesia, MGH, John Ankcorn, Laboratory of Computer Science, MIT

Oral Photomedicine
Nikos Soukas, DDS,PhD Wellman Laboratories of Photomedicine, MGH

Minimally Invasive Thoracic Surgery – Limits and Frontiers
John Wain, MD Chief of Thoracic Surgery, MGH

The Fate of the OR of the Future
Steve Schwaitzberg, MD Director of Surgical Research, New England Medical Center

Technology for Medicine at Draper Laboratory
Paul Blasche, PhD, DL

CIMIT Program Review #6: Tissue Engineering
Joseph P. Vacanti, MD, John Homans Professor of Surgery, HMS and Massachusetts General Hospital, Team Leader, CIMIT Tissue Engineering/Organ Replacement Program; **Design** – Mohammad Kaazempur-Mofrad, PhD – Massachusetts General Hospital Laboratory for Tissue Engineering and Organ Fabrication, **Microfabrication** – Jeffrey T. Borenstein, PhD – MEMS Fabrication Manager, Draper Laboratory, **Living Channels and Flow** - Hidetomi Terai, MD – Massachusetts General Hospital Laboratory for Tissue Engineering and Organ Fabrication, **Gene Activation in Tissue Engineered Liver** – Erin Ochoa, MD – Massachusetts General Hospital Laboratory for Tissue Engineering and Organ Fabrication, **Liver Tissue Generation** – Kohei Ogawa, MD - Laboratory for Tissue Engineering and Organ Fabrication,
Tissue Engineering and Tumor Regression – Antonia Stephen, MD – Massachusetts General Hospital Laboratory for Tissue Engineering and Organ Fabrication, Vascular Tissue Engineering – Irina Pomerantseva, MD - Laboratory for Tissue Engineering and Organ Fabrication, **A Biologic Tooth** – Pamela Yelick, PhD - The Forsyth Institute, **Fetal Tissue Engineering** - Julie Fuchs, MD - Laboratory for Tissue Engineering and Organ Fabrication, **Tissue Engineering and Art** – Oron Catts - Laboratory for Tissue Engineering and Organ Fabrication; Ionat Zurr - Laboratory for Tissue Engineering and Organ Fabrication

Design of Coronary Stents from Engineering First Principles
Kobi Richter, PhD, President, Medinol

Evaluating Stent Performance and Biocompatibility in Bench and Animal Models
Campbell Rogers, MD, Brigham & Women's Hospital, Elazer Edelman, MD, PhD, Brigham & Women's Hospital, Massachusetts Institute of Technology

Gaining Clinical Approval
Donald Baim, MD, Brigham & Women's Hospital, CIMIT

Fire and Ice: In-Situ Thermal Ablation of Liver Tumors
Scott Gazelle, MD, PhD, Massachusetts General Hospital; "Overview of the clinical problem - the surgeon's perspective" Kenneth Tanabe, MD, Chief of Surgical Oncology, Massachusetts General Hospital; "Applications and Results of Ablation Using Radiofrequency Energy" Nahum Goldberg, MD, Beth Israel Deaconess Medical Center; "Applications and Results of ablation using cryotherapy" Stuart Silverman, MD, Brigham & Women's Hospital

Frontiers in Space Medicine

Tom Marshburn, MD, NASA Flight Surgeon and Jeff Sutton, MD, PhD, Team Lead, Smart Medical Systems, NSBRI - Neural Systems Group and Department of Emergency Medicine, Massachusetts General Hospital, Harvard / MIT Division of Health Sciences and Technology

Medical Device Innovation at Stanford
Paul G. Yock, MD, Martha Meier Weiland Professor, Stanford School of Medicine, Director of the Center for Research in Cardiovascular Interventions at Stanford

Deterrents to Innovation with Emphasis on Confusion over Conflict of Interest Issues
Thomas J. Fogarty, MD, Professor of Surgery at Stanford University; Panel: Jonathan Rosen, Ronald Newbower, James Muller, Fiona Murray

Robotic Cardiac Surgery
Lawrence H. Cohn, MD, Virginia and James Hubbard Professor of Surgery, Harvard Medical School, Chief of Division of Cardiac Surgery, Brigham and Women's Hospital; David C. Brooks, MD, General and GI Surgery, Brigham and Women's Hospital; Rob Howe, David Rattner, MD, Keith B. Isaacson, MD

Peer Review at NIH
Lee A. Rosen, PhD, Scientific Review Administrator for the Diagnostic Imaging Study Section, NIH

CIMIT Program Review #7: Vulnerable Plaque Program
James Muller, MD, Massachusetts General Hospital, CIMIT; Thomas J. Brady, MD, Massachusetts General Hospital, CIMIT; Brett Bouma, PhD, Massachusetts General Hospital

New Technologies for Vulnerable Plaque Detection
Walter Koroshetz, MD, Massachusetts General Hospital OCT carotids; Mike Rosol, PhD, Massachusetts General Hospital Volume CT; Kent Yucel, MD, Brigham and Women's Hospital Intravascular MRI; Ahmed Tawakol, MD, Massachusetts General Hospital Preliminary Nuclear Data; Johanna Bosch, PhD, Massachusetts General Hospital Technology Assessment; Panel: Donald Baim, MD, Brigham and Women's Hospital; Mason Freeman, MD, Massachusetts General Hospital, I.K. Jang, MD, Massachusetts General Hospital, Steven Oesterle, MD, Massachusetts General Hospital

Robotically Assisted GI Surgery, 2001
Mark A. Talamini, MD, Associate Professor of Surgery, Johns Hopkins University School Of Medicine and Director of Minimally Invasive Surgery, Johns Hopkins Hospital; David F. Torchiana, MD, Massachusetts General Hospital, David Brooks, MD, Massachusetts General Hospital

Image-Guided HIFU Therapy
Lawrence A. Crum, Director, Center for Industrial and Medical Ultrasound and Research Professor of Bioengineering and Electrical Engineering, University of Washington; Kullervo Hynynen, PhD, Massachusetts General Hospital

Perspectives on Minimally Invasive Surgery from the University Hospital of Trondheim
"National Center for Advanced Laparoscopic Surgery" and "Image Guided Neurosurgery by Intraoperative 3D Ultrasound" Ronald Mårvik, MD, PhD, Department of Surgery, University Hospital of Trondheim; Timothy Brauns, David Rattner, MD, Keith Isaacson, MD, Lee H. Schwamm, MD; "OR of the Future for Minimally Invasive Surgery" Hans Olav Myhre, MD, PhD, Professor and Chairman Department of Surgery, University Hospital of Trondheim

R&D from the Center of Excellence in 3D Ultrasound, Sintef Unimed
"3D Ultrasound in Minimally Invasive Surgery – Product Concepts SonoWand and SonoDoppler" Toril A. Nagelhus Hernes, Research Director; "Postoperative Assessment of Endovascular Procedures using 3D Ultrasound" Jon Harald Kaspersen, PhD, Mechanical Engineering, Research Scientist

Optical Techniques for Epithelial Cancer Detection
Lev T. Perelman, PhD, Beth Israel Deaconess Medical Center, MIT

Clinical Focus Session on Urology
Robert Krane, MD, Director of Neurourology, MGH
W. Scott McDougal, MD, Chairman, Department of Urology, MGH

Role of Force Feedback and Force Control in Medical Robotics
Steve Charles, Memphis, Tennessee

Video Rate 3-D Surface Imaging
Douglas Hart, PhD, D'Arbeloff Associate Professor and Director of MIT Fluid Mechanics Laboratory, Department of Mechanical Engineering, Massachusetts Institute of Technology

The HMC Clinic Program and Minimally Invasive Research Studies
Jon Strauss, PhD, President, Harvey Mudd College
Richard Haskell, PhD, Professor of Physics, Harvey Mudd College

Patient-specific Medical Modeling
Charles Taylor, Stanford University

HCFA, AHRQ, and the Medicare Coverage Decision Process: A Role for Academia?
Sean Tunis, MD, MSc, Director, Coverage and Analysis Group, Health Care Financing Administration
Deborah A. Zarin, MD, Director of the Technology Assessment Program, Agency for Healthcare Research and Quality

Laser Technologies: Advancing State-of-the-Art
Aram Mooradian, PhD, Chief Technology Office, Novalux, Inc.

Panel Discussion

Aram Mooradian, PhD, Chief Technology Office, Novalux, Inc.
Shawn Burke, Photonics Center at Boston University
Rox R. Anderson, MD, Dermatology, Massachusetts General Hospital

Emergency Services Clinical Focus Sessions – 2 Part Session
Alasdair Conn, MGH Chief of Emergency Services
Toby Nagurney , MGH Emergency Services
David Brown, MGH Associate Chief of Emergency Services

Wearable Health
Sandy Pentland, Academic Head, MIT Media Lab

Endovascular Aneurysm Repair: Present Hurdles and Future Possibilities
Richard A. Baum, MD, Assistant Professor, Angiography, UPHS

Molecular Analysis Technologies for Cancer: Tools for Today and Tomorrow
Carol A. Dahl, PhD, Director, Office of Technology and Industrial Relations National Cancer Institute

Advanced Medical Diagnostics: From Lasers to Biochips
Tuan Vo Dinh, PhD, Group Leader, Corporate Fellow, Advanced Monitoring Development Group, Oak Ridge National Laboratory

Future Trends in Minimally Invasive Surgery
Demetrius E.M. Litwin, MD, Associate Professor of Surgery, Director, UMASS EndoSurgery Center, UMass Medical Center

Translational Technology Development and Cancer Research at Harvard
David M. Livingston, MD, Deputy Director, Dana-Farber/Harvard Cancer Center and Chief, The Charles A. Dana Division of Human Cancer Genetics and the Emil Frei Professor of Medicine at Harvard Medical

Segmentation, Modeling and Analysis of Internal Organs
Demitris N. Metaxas, PhD, Associate Professor, Director of VAST Lab (Vision, Analysis and Simulation Technologies Laboratory), Division of Computer and Information Science, University of Pennsylvania

Kinematic Couplings and Elastic Averaging: Methods for Achieving High Precision with Low Cost
John Hart, Graduate Research Assistant, Patrick Willoughby, Graduate Research Assistant, Precision Engineering Research Group, Department of Mechanical Engineering, Massachusetts Institute of Technology

Nanobiotechnology: Interfacing the Physical and Biological Worlds

Michael S. Isaacson, Associate Dean of Research, Graduate Studies and Professional Education, Professor of Applied and Engineering Physics, Director, W.M. Keck Program in Nanobiotechnology, College of Engineering, Cornell University

Applications of MEMS Technology in Chemistry, Biology and Medicine at the Draper Laboratory
Jeffrey Borenstein, MD, Group Leader, MEMS Technology, Charles Stark Draper Laboratory
Chris Dubé, PhD, Senior Member of the Technical Staff, Micromechanical Sensor Development, Charles Stark Draper Laboratory
Angela Zapata, PhD, Senior Member of the Technical Staff, Micromechanical Sensor Development, Charles Stark Draper Laboratory, Group Leader, MEMS Technology

The Opportunities of Software Agents in Healthcare
Patty Maes, Associate Professor, Media Lab, Massachusetts Institute of Technology

Cyber Care
Joseph M. Rosen, MD, Adjunct Associate Professor and Lecturer, Dartmouth Thayer School and Associate Professor of Surgery, Dartmouth Hitchcock Medical Center

SurgON: A Communications Standard for Information Flow and Device Control in the Operating Room
Richard Bucholz, MD, FACS, Professor of Neurological Surgery, Saint Louis University School of Medicine Surgery

Center For Future Health: Creating a Consumer Health Infrastructure
Alice P. Pentland, MD , James H. Sterner Professor, Chair of Department of Dermatology, University of Rochester

A Photochemical Method for Tissue Adhesion
Robert W. Redmond, PhD, Associate Professor, Wellman Laboratories of Photomedicine, Massachusetts General Hospital

Low Cost High Precision Track-Based Mobile Robot Concept for Hospital Automation?
Alexander H. Slocum, Professor, MIT
Shorya Awtar, PhD Candidate, Precision Engineering Research Group, MIT

A Pre-Hospital Diagnostic System For Trauma Victims In A Battlefield Environment
Harvey Mudd College Engineering Team – Vorapat Chowanagisai, Daniel Chin, Ronalee Lo, Emily Williams

The New National Institute Of Biomedical Imaging And Bioengineering
Stanley Baum, University of Pennsylvania

New Methods for Making Medical Products Fit Their Users
Stephen Wilcox, PhD, Design Science, Philadelphia, PA

Optics in Medicine Lectures
Brett Bouma, PhD, Assistant Professor, Dermatology, MGH
Gary J. Tearney, MD, PhD, Pathology, MGH

Image-Guided Cardiac Surgery: Possibilities for Now and the Future
Stephen W. Downing, MD FACC, FACS, Assistant Professor of Surgery, Division of Cardiac Surgery, University of Maryland Medical Center

Computer Aided Detection of Lung Cancer: Computer Vision to the Rescue
Joseph Mundy, PhD, Image Understanding Lab, GE Corporate Research and Development

VIRGIL: A Simulator Training System for Chest Trauma
Steve Dawson, MD, Team Leader, Procedural Simulation, CIMIT

CIMIT Forum Brainstorming Meeting

APPENDIX B: CIMIT Regulatory Affairs Initiative
Program Director: John J. Smith, MD, JD, MGH

Maximum clinical impact of safe and effective new medical technologies is heavily dependent on timely Food and Drug Administration (FDA) marketing approval and third-party payer coverage/reimbursement. The Regulatory Affairs Program at CIMIT provides a unique, national resource to address regulatory and coverage/reimbursement challenges throughout the product development lifecycle.

During the 2001 fiscal year, the Regulatory Affairs Program remained focused on making the existing regulatory and coverage/reimbursement system more efficient, transparent and predictable. Efforts on developing a workable paradigm for "least burdensome means" continue with FDA. A new dialogue with industry and Congress has also focused on this issue, as well as other related matters driven by the Food and Drug Administration Modernization Act of 1997 (FDAMA). High level discussions with the Centers for Medicare and Medicaid Services (CMS) on the Medicare coverage process also continues, as do parallel talks with industry. Finally, research has begun in earnest on conflict of interest in medical research in conjunction with Stanford University.

Key Results:

- Assisting FDA with in implementing the "least burdensome means" concept as required by the Food and Drug Administration Modernization Act of 1997 (FDAMA). The Regulatory Affairs Program has functioned as a point of access to FDA's Center for Device and Radiological Health, providing information and academic resources to FDA managers and line reviewers.
- Co-sponsorship of a summit on conflict of interest in medical research with Stanford University. This successful meeting, conducted in July 2001 at Stanford, is envisioned as the initial step of an ongoing collaborative effort to address this important issue.
- High-level meetings with officials of CMS' Coverage and Analysis Group (CAG). In March and again in July 2001, Sean Tunis, M.D., CAG Director, visited CIMIT with his staff. The initial visit was Dr. Tunis' introduction to our organization, while the second meeting was at Dr. Tunis' request, designed to begin a thoughtful process to increase the transparency and efficiency of the Medicare coverage process.
- CIMIT White Papers on FDA Regulation of the Reuse of Single Use Medical Devices and Medicare's new Hospital Outpatient Prospective Payment System. Neutral, scholarly analysis of these important topics were released during the 2001 fiscal year.
- Scholarly publications in medical and legal literature. Research directed at identified regulatory and coverage/reimbursement issues has resulted in numerous published and accepted articles, with additional articles submitted for consideration for publication.

Specific Aim 1: Maintain an infrastructure for addressing systemic regulatory and reimbursement issues in device development, as well as product-specific questions that arise within CIMIT investigators and its industrial collaborators.

Progress: The Regulatory Affairs Program maintains a robust capacity to identify and assess systemic issues in medical device regulation and coverage/reimbursement. Methods include

regular discussions with industry, government, and the medical community, as well as tracking of publicly available print and web-based resources. This material serves as the foundation for a monthly regulatory newsletter, broadly available to the CIMIT community.

In addition to the focus on systemic issues, the Regulatory Affairs Program has provided CIMIT investigators with product-specific regulatory and coverage/reimbursement assistance on an as-needed basis, generally in coordination with CIMIT's Office of Technology Development. This assistance is intended to aid investigators engaged in early-stage research, not as a supplement or replacement for industry-provided support in projects with industry involvement. In fiscal year 2001, the Program aided the Office of Technology Development in developing the regulatory section of a CIMIT Investigator's Handbook and assisted that office with a case-by-case regulatory review of currently funded CIMIT projects.

Plan: The Regulatory Affairs Program will continue to identify and assess systemic issues using current methods, using the results to guide research and produce the month Regulatory Newsletter. In addition, product-specific regulatory and coverage/reimbursement services will continue to be offered to CIMIT investigators, in close cooperation with the Office of Technology Development.

Specific Aim 2: Identify key regulatory and coverage/reimbursement issues facing the device development process across product lines ("systemic" issues).

Progress: An ongoing dialogue with government and industry to identify and define these issues has been established. Defining "least burdensome means" under the Food and Drug Administration Modernization Act of 1997 (FDAMA) and FDA's revised policy on the reprocessing and re-use of single-use medical devices remain important issues, with ever increasing attention focusing on the federal regulation of conflict of interest in clinical trials.

In the coverage/reimbursement area, industry and government appear increasingly ready for major change in the management of the Medicare program. CMS appears commitment to the development of a more coherent coverage process, a policy with considerable implications for industry. A second important issue is the ongoing implementation of CMS' new hospital outpatient prospective payment system.

Plan: FDA implementation of "least burdensome means" is increasing focused on its actual application to 510(k) and PMA applications, an area where the agency concedes its need for cutting-edge scientific and clinical expertise. Through the Regulatory Affairs Program, CIMIT has provided FDA with resources on stroke and vulnerable plaque. This efforts will continue, and hopefully, expand in the near future.

A successful meeting with Sean Tunis, M.D., Director of CMS's Coverage and Analysis Group, and his staff was held in mid-July 2001, the first of a planned series of meetings to explore current coverage models and effect positive change. FDA, at the highest levels, has indicated an interest in becoming involved in this dialogue. A more informal dialogue with industry has confirmed that sectors' continuing interest in this initiative. Work to move these various initiatives forward will continue.

Specific Aim 3: Develop and apply a process for developing workable solutions to regulatory and reimbursement issues.

Progress: The Regulatory Affairs Program regulatory and coverage/reimbursement problem-solving process continues its use of three interrelated mechanisms to effect positive change: (1) objective, scholarly white papers to serve as an informational resource and basis for further action; (2) innovative solutions crafted in collaboration with interested stakeholders; (3) CIMIT-sponsored forums directed at specific issues.

During the 2001 fiscal year, work continued on scholarly publications linked to completed research on FDA's new regulatory scheme for the reprocessing and re-use of single-use medical devices, as well as CMS' recently implemented hospital outpatient prospective payment system.

Innovative solutions to systemic issues continue to receive attention. CMS, in particular, has become interested in CIMIT-sponsored forums to more clearly delineate issues with the Medicare coverage and payment process, and to work towards solutions. FDA appears interested in our work in this area as well, and has indicated a willingness to become more directly involved on coverage and payment efforts, as they impact on medical technology regulation.

Responding to a perceived need in the academic medical community and industry, the CIMIT Regulatory Affairs Program has joined with Stanford University to explore federal regulation of conflict of interest in medical research. The first result of this collaboration, a forum at Stanford University, successfully delineated issues associated with conflict of interest in the academic medical setting.

Plan: Planning for a Washington, D.C., based retreat to follow-up on the successful July 2001 meeting with CMS on coverage issues will continue, as will efforts to engage FDA in this process. Discussions with Stanford officials on a follow-up Boston-based forum discuss potential solutions to the conflict of interest issue are underway, with a meeting expect in Spring 2002. In addition, scholarly research directed at this issue is well underway, with a white paper expected in the near future.

APPENDIX C: Operating Room of the Future and APRIL

Program Directors: David Rattner, MD and Reuben Mezrich, MD, PhD

The MGH and CIMIT are initiating a collaborative effort with industry to build out a state of the art operating room that will serve as a Beta Site for newly approved, state of the art surgical and information systems technology. Industry is contributing equipment and establishing a collaborative joint agreement for this project. The goal is to create an operating suite for the evaluation of novel technology intended to improve OR efficiency and patient safety, prior to dissemination across multiple operating rooms. This project will include developing models to demonstrate the impact of a new technology on time, costs, staffing and flow. Specific focus as the room is opened in September 2001 will be to implement technology that will:

- Enable optimum interaction of new technologies of various equipment, enhancing overall efficiency of the OR
- Improve the ability to know the location of patients and equipment
- Improve operating room ergonomics
- Improve disposable inventory management
- Enhance patient flow / decrease turnover time
- Enhance anesthesia efficiency including anesthesia induction in induction room adjacent to the OR
- Enable networked connectivity (including wireless, where appropriate) between medical devices
- Efficiently educate and credential end-users in the safe and proper use of the innovative patient care devices

The committee of the whole has met weekly, comprised of architects, physicians, nurses, operating room administration, information systems representatives, and CIMIT leadership. Several subcommittees address particular areas, such as equipment networking. The implantation of a beta site room to test and trial new devices prior to broad purchase or use is seen as a new model of merit.

APRIL - Advanced Procedure Room and Innovation Laboratory

A group of physicians from the Massachusetts General Hospital and the Brigham and Women's Hospital, as well as engineers and scientists from Massachusetts Institute of Technology continue to work on developing the operating room technology of the future. This project, whose acronym APRIL stands for the Advanced Procedure Room and Innovation Laboratory, has a goal of bringing together leading researchers and practitioners from the fields of computer science, engineering, architecture and medicine to create a better environment and better tools for the delivery of minimally invasive surgical procedures. The work has been divided into four major areas: Computer assisted surgery; data capture and analysis; facility design and infrastructure, and software and hardware architectures for advanced wireless "patient-area-networks" of sensors and actuators. Proposals for funded research in these areas are presently being received and reviewed. The goal is to fund new concepts in this area, and then refund appropriate efforts that show promise. The build out of an alpha operating room laboratory

within the area of CIMIT where the medical simulation and device simulation work will be co-located as of August allows for a true test site for this work to be conducted.

Progress This Year

Operating Room of the Future (ORF) Implementation at MGH
Dr. Keith Isaacson, the Project Leader, and members of the design team presented plans and status to the Executive Committee of the Hospital and were given approval to proceed with construction. The best estimate of time for beneficial occupancy of the room is now September 15, 2001 which represents a delay of approximately one month.

The final room area has been cleared, and full size mockups are now being built to check physical clearances for major equipment. A computer-based simulation has resulted in the development of an animated video that shows patient flow, equipment positioning, and some details of equipment racks dedicated to specialized procedures. This video has been made available to the team as a Compact Disc, and has proved useful in communicating the project goals as well as the details of room utilization.

The specifications for major equipment subsystems have been made firm, details are now being determined for facilities support and interconnections.

Contracts with industry partners are becoming firm, with all but two of the primary vendors having signed agreements to collaborate. One industry partner (Pinpoint, which was to provide wireless sensing of equipment locations) has dropped out; a replacement is being sought.

Cost Effectiveness of New OR Configurations
(Technology Assessment Program – Scott Gazelle, MD, Program Leader)

Prior to investigating the potential benefit of innovations planned as part of the OR of the Future Project, it was necessary to understand the current or "base case" environment. For this analysis, a discrete event simulation model was developed. This base case model was specifically developed in order to incorporate technologies and processes which have been targeted for change in the ORF. This detailed model of the current system will be used to generate benchmarks against which new technologies and processes may be evaluated. The base case modeling effort may also help to identify specific operating inefficiencies in the current system, and to determine where ORF innovations may be the most effective.

Thus far, the model has been used to test some of the basic staffing hypotheses of the ORF project. For example, it was hypothesized that making anesthesia coverage modular from induction, through the operative procedure, to post operative recovery would result in productivity gains over the current, tightly coupled system where a single anesthesia team monitors the patient continuously from induction through recovery. Using process flow information from the current surgical environment, the team compared different approaches to process flow in the operating room.

The preliminary analysis compared three different scenarios: 1) the current OR system with 2 nurses, 1 anesthesiologist and one surgeon; 2) a second scenario with 2 nurses, 2 anesthesiologists and one surgeon; and 3) and the proposed ORF system with 2 nurses, 2 anesthesiologists and one surgeon. These three scenarios were compared with respect to patient throughput, nursing, anesthesiology and overall OR utilization, and estimated costs per year for routine laparoscopic cholecystectomy.

Results of Preliminary Analysis

	Current OR		**2nd Scenario**		**Proposed ORF**	
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***
Patients Per Day	4.2	.42	4.2	.42	6	.99
Percent Utilization*						
OR	45%	6%	45%	6%	72%	4%
RN	97%	5%	97%	5%	68%	4%
Anesthesia	93%	6%	46%	3%	43%	2%
Ave. Daily Cost (± S.D.)	$26,191(±3158)		$26,338(±3159)		$23,118(±4279)	

* percent utilization = (actual utilization)/(total possible utilization)

These data demonstrate that the proposed ORF would result in a 43% increase in patient throughput and a 60% increase in OR utilization while at the same time reducing utilization of nursing and anesthesia staff. These differences were statistically significant ($p < 0.05$). Average costs would decrease by approximately 12%, though this difference was not significant (possibly due to the small number of simulations performed).

Wireless Connectivity

A specific goal of the ORF Project is to enhance the understanding of the role of wireless device connectivity in operating room environments. These environments represent unique challenges due to increasing simultaneous use of various energy-delivery and imaging devices. The Project will leverage the results of a sophisticated study of wireless interference being conducted (in a "general care" area of the Hospital) with industry cooperation at MGH by Partners Healthcare Information Services in collaboration with Nat Sims, M.D. of Biomedical Engineering and Michael Dempsey, formerly Chief Wireless Strategist for Agilent Healthcare. In particular, a heavily-loaded wireless network (802.11 Frequency-Hopping) of devices transmitting critical continuous physiological waveforms will be stressed by exposure to other wireless devices transmitting data using 802.11 Direct Sequence and/or Bluetooth modulation schemes.

Web-Based e-Learning

Another key goal of the project is to ensure that experience is gained with advanced-technology web-based e-learning-systems for the efficient education and certification of end-users, as novel advanced therapeutic technologies enter the operating room workplace in increasing numbers. As O.R. personnel become advanced knowledge workers, learning systems must rise to the challenge. In anticipation Dr. Sims of MGH launched, collaboratively with GE Medical Systems, in early 2000, a pilot program to repurpose conventional training for a common GE

product offering (ICU physiological monitor) to the web. A sophisticated suite of back-office functions keeps track of users and offers certification and CME's. Thus far the project has been highly successful and will be hard-launched in May 2001. CIMIT is entering discussions with GE about extending this promising technology to innovations in the Operating Room.

Networking in the OR

The ORF team has planned to implement networked connectivity between a host of medical devices (including surgical instrumentation and patient care devices) to facilitate remote view, remote control, and data capture. An example and model of this is represented by the Karl Stortz OR-1 Network, which uses proprietary software drivers and a serial communication network for a limited suite of Stortz and 'other-vendor' instruments (insufflators, light sources and the like). It was envisioned that the 'critical mass' and leadership of CIMIT, Partners, DOD, MIT and others would be catalytic for agreement upon a more-open standard along the lines of UpnP ("universal plug and play") standards being implemented for mass- and home- markets. It is now recognized that achievement of this will be arduous for a variety of reasons. CIMIT is reevaluating its strategy for facilitating this needed development.

Summary

The ORF Management Team is mindful of the risks inherent in rapidly creating a site at a major healthcare institution that attempts to be futuristic, luminary, efficient, flexible, scalable, cost-effective, safe for patients, smoothly integrated with the routine functioning of a busy contiguous 52-room existing facility, and yet adapted for continuous iteration and 'in-transfer' of innovation over a three-year term. The team recognizes that its significant challenge is to find appropriate balance between innovation, achievability, complexity and parsimony, and to deliver a total system that is better for patients and also within the capability of our knowledge workers safely to adapt to change. Thus the hallmark of success may, paradoxically, be a quiet, rather lean and elegantly-simple room with fewer-than usual workers! Other key indicators of success will be the fidelity with which the original design is replicated in subsequent rolling renovations at Partners, the optimized patient-throughput per room, and the optimized labor content per case going forward.

APRIL Project Progress Summary

In the APRIL project, the central effort is going forward under the leadership of Dr. John Guttag of MIT's Laboratory of Computer Science (LCS), and Drs Reuben Mezrich and Nat Sims of MGH/CIMIT. A "Medical Innovation" course, bringing leading researchers and clinicians from Partners Healthcare to lecture weekly at LCS, led to the formation of the "O.R. Net Working Group," comprising 5 MGH/MIT faculty and a group of doctoral students. The overall effort around which the group has aggregated is a radical (but rapidly achievable) concept - a network of independent, wirelessly-connected sensors and actuators in a 'patient-area' network. As of 1 April, 2000, hardware is in development for a reduced-to-practice network using four common physiological sensors and one actuator as exemplary instantiations. A related project is the development of a novel wireless biosensor based on advanced acoustic sensing means and digital signal processing. This acoustic sensor may have applications outside the operating room environment, possibly as an implantable sensor for use in other health environments. Recent progress of the group, including the ORNet Design Specification white paper, is given at http://nms.lcs.mit.edu/~gch/ornet/ with the username "ornet" and password "ornet01".

Appendix D: INDUSTRY COLLABORATIONS PROGRAM

Principal Investigator: Janice E. Crosby, RN, MBA

Collaboration is the cornerstone of CIMIT's success. By uniting researchers, clinicians, industry, and government, CIMIT is overcoming the obstacles that too often keep an innovative idea from becoming a useful product. Eliminating such obstacles is especially important in the fields that contribute to minimally invasive approaches. Stunning advances in areas such as robotics, fiberoptics, imaging, and biomechanical engineering promise cost-effective, less traumatic new therapies, with obvious benefits to both patients and society.

Getting the best of these ideas to market is one of CIMIT's goals. The consortium is committed to supporting promising ideas in the early development and proof-of-concept stages, then assisting in the transfer of the most promising projects to commercial organizations capable of developing successful products.

CIMIT's Business Development Group is the consortium's arm most directly responsible for forming alliances with industry partners. Over the past year, accomplishments of the Group have included:

- Expanding its Industry Liaison Program, meeting with more than 65 companies to establish the groundwork for collaborations. Currently, 29 companies are collaborating with CIMIT, providing $6.3 million through fees, in-kind contributions, and sponsored research.
- Hosting an Annual Stakeholders Briefing, a networking opportunity during which more than 150 attendees were introduced to some of the most promising MIT-based collaborations and saw exciting demonstrations of CIMIT's work in a "science fair" setting.
- Hosting spring and fall meetings with CIMIT's Industry Advisory Board (IAB), a panel of 11 industry leaders who provide valuable insight and direction to CIMIT's leadership. The spring meeting provided a forum for discussing CIMIT's five-year plan in light of future trends in the technology development industry. The fall meeting focused on the status of CIMIT's industry programs and research portfolio and on CIMIT's National Response Plan, designed to fast track or redirect and enhance CIMIT's current activities in order to provide deliverables to improve national crisis response and treatment.

Industry Members include:
John Abele, Founder and Chairman, Boston Scientific Corporation
Paul Citron, Vice President, Science and Technology, Medtronic, Inc
Cynthia Danaher, Industry Consultant
Ronald Dollens, President and CEO, Guidant Corporation
Scott Donnelly, Vice President, Globel Technology Operations, GE Medical Systems
Alexander d'Arbeloff, Chairman, MIT Corporation; and Founder, Teradyne, Inc.
George Rabstejnek, Vice Chairman, Massachusetts Eye and Ear Infirmary
Frank Samuel, Technology Consultant, Office of the Governor, Ohio
Tom Sommer, President, Mass Medic
John Thompson, Attorney, Nutter, McLennan and Fish, LLP
Josh Tolkoff, President, Seedling Enterprises, LLC

- Developing CIMIT's website, which is now a major resource for communicating with both internal and external audiences

The most common way industry partners become involved in CIMIT is by joining the Industry Liaison Program. When companies become ILP members, they gain access to an impressive interdisciplinary organization dedicated to improving patient care by catalyzing the development of innovative technology. Industry can customize its level of participation, from consulting with individual experts to collaborating directly on CIMIT's Programs. CIMIT assigns an industrial liaison to assess the company's needs, define the ideal level of participation, and then manage the relationship to make sure that goals are met.

Industry sponsorship was also actively solicited for the CIMIT/MGH project to build out the Operating Room of the Future (ORF) and CIMIT's program on Vulnerable Plaque (VPP). At present, 10 companies are participating in the ORF Program, all of whom share CIMIT's vision and best meet the needs for surgical equipment, booms and lighting, patient transport, energy delivery, anesthesia delivery, physiologic monitoring, infusion management, personnel tracking, and inventory tracking and storage. In addition, eight companies have become sponsors of the Vulnerable Plaque Diagnosis and Treatment Program.

A more intense level of interaction involves sponsored research to meet specific goals of mutual interest. CIMIT can provide the optimal environment in which to work, helping to coordinate the key clinical and technology teams from multiple specialties to conduct corporate sponsored research.

The most serious, ongoing level of mutual commitment, suitable for large companies with major technological resources and a significant presence in their market, is that of "Strategic Alliance Partner". CIMIT's goal is to establish a limited number of such partnerships, wherein each party contributes, at their own expense, expertise in problem solving, research, development, prototyping, and feasibility engineering. Industry will provide the equipment and CIMIT will provide technical and clinical evaluations and an assessment of the clinical and financial impact of the new technology. The team envisions 5-10 major companies becoming members of the CIMIT Strategic Alliance.

Underlying all these interactions are carefully crafted principles protecting intellectual property and the fair and appropriate assignment of rights to inventions. At CIMIT, attention is devoted to tracking intellectual property brought to the collaboration by partners and IP developed within the conduct of CIMIT projects.

Currently, the following companies are collaborating with CIMIT:

AstraZeneca, LP
Baxter Healthcare Corp.
Boston Scientific Corp.
Compaq Computer Corp.
Draeger Medical Corp.

EndoVasix, Inc.
GE Medical Systems, Inc.
Getinge-Castle, Inc.
Guidant Corp.
Harvard Clinical Technologies, Inc.
Imetrx, Inc.
InfraReDx, Inc.
Johnson & Johnson
    Cordis Corp.
    Ethicon Endo-Surgery, Inc.
    Mitek Products
Karl Storz Endoscopy-America, Inc.
Medtronic, Inc.
Mobile Aspects, Inc.
Omnicell, Inc.
Onux Medical, Inc.
Pentax Precision Instrument Corp.
Percardia, Inc.
Pfizer, Inc.
Pharmacia, Inc.
Sentinel Wireless, Inc.
Siemens Medical Systems, Inc.
TherOx, Inc.
TissueLink Medical, Inc.
Welch Allyn, Inc.

Appendix E: Communications Program/PR
Principal Investigator: Janice E. Crosby, RN, MBA

CIMIT has designed a communications program to address its diverse communication needs, audiences, and resources. Key objectives include: educating researchers and clinicians about the potential of new applications of technology to meet medical needs and establishing CIMIT as the preeminent resource on the development and integration of technology into Medicine.

CIMIT has decided to utilize the powerful tool of the Internet to promote the CIMIT mission and the programs and participants that compose the organization. By taking advantage of the internet and database technologies, the CIMIT websites become useful and engaging tools not just for the general information seeker, but for those who want to actively participate in CIMIT. Building onto the newly designed public website, the CIMIT web team has developed and implemented an expansive, **web-based knowledge management system** for members and staff called "myCIMIT".

In the first phase, CIMIT designed and built a SQL database infrastructure for myCIMIT between January and May 2001. This secure site provides information to the member about his/her "account," i.e., specific meeting agendas and minutes, company agreements and project proposals, and contacts within the CIMIT organization. On the back-end, administrators of the site and some staff members can access a multitude of reports, input documents and media files, and can manage user information and access privileges. This initial phase of myCIMIT was by far the most lengthy, requiring many hours of planning and testing in order to provide a solid foundation for future enhancements.

To adequately supply archives of our weekly CIMIT forum to members and CIMIT staff, a feature the CIMIT community has longed to have, the next phase for the myCIMIT site was to develop the CIMIT FORUM section of the site. Between July and August 2001, CIMIT created a searchable archive of over 90 forum sessions, including text summaries, videos, and PowerPoint presentation files. Users can search the archives by keyword, dates, and even by speaker name. Remote access to the CIMIT FORUMs is important to the various stakeholders who are unable to attend these meetings.

Although the myCIMIT site has provided a great repository for vital information for both CIMIT staff and members, CIMIT needs to focus on fine tuning the site so that the display of information is more easily manipulated, the navigation is more intuitive, and data is presented effectively. CIMIT wants to more effectively utilize the power of this database infrastructure so that not only can a user access information and documentation on a particular project, program, contact, meeting, or agreement, but he/she can even exchange documents or view videos while conducting an online project meeting or participating in a CIMIT forum. Next steps also include the development of a Vulnerable Plaque Program site, and a site for the Office of Technology Development. As the team moves forward with enhancements and additional program sites, tapping into a central, robust database structure will minimize the duplication of effort and maximize the exchange of knowledge.

The core of CIMIT's mission is collaboration, and utilizing the web to bring together the experts who are key to accelerating the technology development process is the best means to that end. The team hopes these and many other developments will break down the barriers of distance, time, and money.

Additional communication programs for CIMIT include:

- Publication of CIMIT's 2000 Annual Report
- Annual Stakeholders Briefings that highlight some of the CIMIT collaborations and research portfolio
- Participation and presentations at National forums in such areas as Conflict of Interest, Industry meetings i.e. Mass Medic etc.

In its role as a convenor, CIMIT is preparing CDs of the various conferences it supports. The first such offering will be for the Vulnerable Plaque Scientific Conference in October 2001. The goal is to disseminate the presentations and discussions of the assembled world leaders on the pathophysiology, detection and treatment of Vulnerable Plaque.

APPENDIX F. LIST OF CIMIT PROJECTS AND PRINCIPAL INVESTIGATORS

CIMIT RESEARCH PROJECTS

ENDOVASCULAR DEVICES

Cardiomyocyte Repopulation using Percutaneous Delivery of Tissue Engineered Systems
Stephen Oesterle, MD MGH

MINIMALLY INVASIVE SURGERY

Minimally Invasive Cardiac Surgery – Endoscopic Coronary Anastomosis
David Torchiana, MD MGH

Endothelial Activation Markers as Molecular Targets for Innovative, Minimally Invasive Diagnosis and Therapy in Cardiovascular Disease
Michael Gimbrone, MD BWH

Develop a Computer-Based Three-Dimensional Imaging Treatment Planning System to Drive an Endoscopically Placed, Miniature, Facial Skeletal Distraction Device
Leonard B. Kaban, MD, DMD and Maria Troulis, MD, MGH

IMAGE GUIDED THERAPY

MRI-guided Focused Ultrasound Treatment of Breast Cancer
Ferenc Jolesz, MD BWH

Early Detection and Ablation of Epithelial Cancers
Norman Nishioka, MD MGH

Segmentation of Bone From CT and Vessels From MRA Data
Carl-Fredrik Westin, PhD and Ron Kikinis, MD, BWH

Real-time Registration of Intra-operative Ultrasound with Pre-operative CT/MR for Image Guided Therapy
Eric Grimson, PhD MIT

TISSUE ENGINEERING

Degradable Conductive Polymers
Robert Langer, ScD MIT

Polymer-based Gene Delivery Platform
Robert Langer, ScD MIT

Transdermal Drug Delivery and Chemical Sensing for Neonates Using Skin Electroporation
James Weaver, PhD MIT

Synthesize Vascularized Living Systems from the Platform of Two-Dimensional Silicon Microfabrication Technologies and Adapt to
Three-Dimensional Living Devices
Joseph Vacanti, MD MGH

Synthesize Vascularized Living Systems from the Platform of Three-Dimensional Printing Technology
Joseph Vacanti, MD MGH and Jeffrey Borenstein, PhD, Draper Laboratory

Minimally Invasive Meniscal Repair with Tissue Engineered Cartilage
Thomas J. Gill, MD and David J. Zaleske, MD, MGH

Development of a Novel *in vivo* Recombinant Protein Delivery Device Designed to Regress Abnormal Tissue: Recombinant Human Müllerian Inhibiting
Substance (rhMIS) Producing Cells on Biodegradable Matrices
David MacLaughlin, PhD MGH

Determine the Role of Mesenchymal Stem Cells in Fetal Tissue Engineering
N. Scott Adzick, MD, University of Pennsylvania (UPenn) and Children's Hospital of Philadelphia (CHOP) and Alan W. Flake, MD, CHOP

SIMULATION

Design, Fabricate and Validate Procedural Medical Simulators
Steven L. Dawson, MD MGH

NEW INITIATIVES

Lung Volume Reduction Using a Bronchoscopic Approach
Edward Ingenito, MD BWH

Outcome Assessment in Menorrhagia
Johana L. Bosch, PhD MGH

Development of Fiberguides for Use in Medical Laser Applications
Vivek Reddy, MD MGH

TRAUMA AND CRITICAL CARE

Microsensors – Real-Time Blood Assay
Christopher Dube, PhD Draper Laboratory

Application of Microwave Imaging to Rapid Non-Invasive Detection of Intracranial Hematoma
Geoffrey Ling, MD, PhD Uniformed Services University of the Health Sciences, USUHS, Bethesda, MD

Near-Infrared Reflectance Spectroscopy (NIRS) to Assess Regional Ischemia both during Trauma Resuscitation and at the Bedside in the Intensive Care Unit
Juan Carlos Puyana, MD BWH

Noise-Enhanced Tactile Sensation for the Management of Sensory Deficits in Patients with Stroke
Casey Kerrigan, MD Spaulding Rehabilitation Hospital

VULNERABLE PLAQUE

Detection of Vulnerable Plaque using Optical Coherence Tomography
Brett Bouma, PhD MGH

Coronary Angioscopy for Detection of Vulnerable Plaques
IK-Kyung Jang, MD MGH

STROKE

Acute Stroke Management – Neuro-Protection
Walter Koroshetz, MD MGH

MRI Guided Rapid Laser Endovascular Photoacoustic Recanalization (LEPAR) for Hyperacute Stroke and Stroke Predictive Modeling
R. Gilberto Gonzalez, MD, PhD MGH

Optical Monitoring and Imaging of Stroke
David Boas, PhD MGH

Neuronal Injury and Neuroprotection in Epilepsy: Proton Beam Radiation for Intractable Epilepsy
Jonathan Brisman, MD MGH

Telemedicine – Remote Stroke Videoconferencing Project
Lee Schwamm, MD MGH

OTHER PROGRAMS

OR of the Future
David Rattner, MD, MGH

Application of a Robotics Interface in Surgery
David Torchiana, MD, MGH

APPENDIX G: LIST OF PERSONNEL RECEIVING PAY

PROGRAM/PROJECT TITLE	NAME	TITLE/ROLE
CARDIOVASCULAR		
Management	Muller, James, M.D.	Cardiovascular Program Team Leader
	Brady, Thomas, M.D.	Cardiovascular Program Team Leader
	Ryan, Jeanne	Administrative Assistant
OCT for plaque characterization	Bouma, Brett E., Ph.D.	Principal Investigator
	Jang, Ik-Kyung, M.D., Ph.D.	Investigator
	Brady, Thomas, M.D.	Investigator
	Shishkov, Milen, Ph.D.	Research Fellow
	Kang, Dong-Heon, M.D.	Investigator
	Yabushita, Hiaroshi, M.D.	Investigator
	Schlendorf, Kelly	Clinical Studies Coordinator
Minimally invasive cardiac surgery	Torchiana, David F., M.D.	Principal Investigator
	Howe, Rob, Ph.D.	
	White, Jennifer, M.D.	Research Fellow
	Titus, James	Lab Supervisor
CANCER		
Management	Tanabe, Kenneth, M.D.	Cancer Program Team Leader
NIR spectroscopy-Barrett's esophagus	Nishioka, Norman, M.D.	Principal Investigator
	Schomacker, Kevin, Ph.D.	Co-Investigator
	Brand, Stephan, M.D.	Research Fellow
	Puricelli, William, R.N.	Clinical Coordinator
OCT imaging of esophageal lesions	Nishio0ka, Norman, M.D.	Principal Investigator
	Bouma, Brett, Ph.D.	Co-Investigator
	Asimellis, George, Ph.D.	Research Fellow
	Puricelli, William, R.N.	Clinical Coordinator
MRA & CT Segmentation	Westin, Carol F., Ph.D.	Principal Investigator
STROKE		
Management	Koroshetz, Walter, M.D.	Stroke Program Team Leader
	Schwamm, Lee, M.D.	Stroke Program Team Leader
	Gonzalez, R., Gilberto, M.D., Ph.D.	Stroke Program Team Leader
	Lee, Albert, M.D.	Researcher
Stroke Metrics	Sorensen, Gregory, M.D.	Principal Investigator
Tissue characteristic in	Sorensen, Alma Gregory, M.D.	Principal Investigator

PROGRAM/PROJECT TITLE	NAME	TITLE/ROLE
human acute ischemic stroke		
Assessment neoroprotective brain cooling	Koroshetz, Walter, M.D.	Co-Investigator
	Lee, Albert, M.D.	Research Scientist
Laser thrombolysis of clot	Gonzalez, R. Gilberto, M.D., Ph.D.	Principal Investigator
Diffusion optical tomography brain hemmorhage	Boas, David, M.D.	Principal Investigator
	Koroshetz, Walter, M.D.	Co-Investigator
	O'Donnel, Joan, R.N.	Nurse Coordinator
	Gaudette, Thomas, Ph.D.	Engineer
	Marota, Joseph, M.D.	Medical Staff
	Mandeville, Joseph, M.D.	Medical Staff
Use proton beam rad – tract epilepsy	Brisman, Jonathan, L., M.D.	Principal Investigator
Measure CV reactivity-functional MRI	Greenberg, Steven, M.D., Ph.D.	Principal Investigator
	McKenzie, Sarah, B.A.	Research Assistant
TRAUMA		
Management	Puyana, Juan Carlos, M.D.	Trauma Program Team Leader
	Puyana, Juan Carlos, M.D.	Principal Investigator
NEW INITATIVES	Dube, Christopher, Ph.D.	Principal Investigator
	Calderwood, Steven, M.D.	Principal Investigator
Management	Dawson, Steven, M.D.	New Initiatives Program Team Leader
	Brady, Thomas, M.D.	New Initiatives Program Team Leader
Computer-based distraction device	Kaban, Leonard B. D.M.D., M.D.	Principal Investigator
	Seldin, Edward B., D.M.D., M.D.	Principal Investigator
	Emanuel, David, D.D.S., M.D.	Investigator
ADV TECHNOLOGY TEAMS		
Management	Pien, Homer, Ph.D.	ATT Program Team Leader
	Dawson, Steven, M.D.	ATT Program Team Leader
Management	Vacanti, Joseph, M.D.	ATT Program Team Leader
	Kelly, Miranda	Administrative Assistant
	Oesterle, Steven, M.D.	ATT Program Team Leader
	Harvey, Susan	Administrative Assistant
Tissue Engineering	Vacanti, Joseph, M.D.	Principal Investigator
	Langer, Robert, Sc.D.	Principal Investigator
	Anderson, Rox, M.D.	Investigator

PROGRAM/PROJECT TITLE	NAME	TITLE/ROLE
	Borenstein, Jeffrey, Ph.D.	Principal Investigator
	Kaihara, Satoshi	Surgical Fellow
	Solan, Lalan	Research Technician
	Kohei Ogawa	Surgical Fellow
	Rahul Koka	Technician
	Cathryn Sundback	Post-Doc Fellow
	Mohammad-Reza Kaazempur-Mofrad	Post-Doc Fellow
	Jamie Lien	Student
	Michael Raven	Student
	Peter Linde	Surgical Fellow
	Michio Sato	Surgical Fellow
	Tracy Griksheit	Surgical Fellow
	Julie Fuchs	Surgical Fellow
	Terai Hidetomi	Surgical Fellow
CORE PROGRAMS	Rattner, David, M.D.	Principal Investigator
CIMIT Clinical Programs	Gesner, Charlotte	Administrative Assistant
CIMIT Education Program	Mezrich, Reuben, M.D., Ph.D.	Director
	Shaffer, David, Ph.D.	Director
	Weissbach, Karen	Program Specialist
	Sage, Melanie	Administrative Assistant
	Strod, Deborah	Technology Associate
Technology Assessment Program	Gazelle, Scott, G. Ph.D.	Principal Investigator
	McNaughton-Collins, Mary, M.D.	Outcomes Analyst
	Halpern, Elkan F., Ph.D.	Statistician
	Gleason, Suzanne, Ph.D.	Economist
	Lester, Jessica, M.D.	Research Associate
	McMahon, Pamela, B.S.	Research Associate
	Maddeford, Jennifer	Staff Assistant
CIMIT OTD	Rosen, Jonathan, Ph.D.	Director
CIMIT Strategy	Parrish, John A., M.D.	CIMIT Director
	Nolan, Marybeth	Administrative Assistant
	Shulman, Beth	Secretary
CIMIT Leadership	Brady, Thomas, M.D.	CIMIT Executive Director
	Muller, James, M.D.	CIMIT MGH Director
	Ryan, Jeanne	Administrative Assistant
	Vosburgh, Kirby, Ph.D.	Leadership
	Deutsch, Thomas, Ph.D.	Science Recorder
	Anderson, Rox, M.D.	Leadership

PROGRAM/PROJECT TITLE	NAME	TITLE/ROLE
	Jang, Ik-Kyung, M.D., Ph.D.	Leadership
	Nishioka, Norman, M.D.	CIMIT Awards Program Director
	Cohen, Melissa	Administrative Assistant
	Isaacson, Keith, M.D.	Leadership
	Stiller, Jane	Administrative Assistant
CIMIT Operations	Osborn, Lynne R.	Director of Administration and Finance
	Garber, Kelly	Administrative Assistant
	Herry-Galloway, Michelle	Administrative Assistant
	Robichaud, Annette	Finance Administrator
	Pagett, Jane	Financial Analyst
	Taylor, George	Courier
CIMIT IS	Cho, Unsuk	IS Manager
	Greaves, Kenneth	IS Manager
CIMIT Program Development	Kigin, Colleen	Director of Program Development
	Chandonnet, Grace	Staff Assistant II
	Palumbo, Andrea	Clinical Studies Coordinator
	Schlendorf, Kelly	Clinical Studies Coordinator
	McAuliffe, Daniel	Stoke Program Manager
	Stod, Deborah	Technology Associate
CIMIT Business Development	Crosby, Janice	Director of Industry Liaison Program
	Humphrey, Ann	Industry Account Manager
	Carpenter, Janine	Industry Project Coordinator
	Hanna, Lamees	Industry Program Coordinator
CIMIT FDA Program	Smith, John J., M.D., J.D.	FDA Program Director

Appendix H. GRADUATE DEGREES RESULTING FROM AWARD SUPPORT

Through his work with Dr. Steven L. Dawson, MD and the CIMIT Procedural Simulation Team, Yann Chaumet, successfully completed his Master's thesis in Computer Science at the EUDIL (Ecole Universitaire d'Engenieurs de Lille).